MATHEMATICAL MODELING OF BACTERIAL SPORE POPULATION DYNAMICS FOR DESIGN AND VALIDATION OF ULTRA HIGH TEMPERATURE STERILIZATION PROCESSES

By

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Major Department: Agricultural Engineering

Objectives of this research were to develop and test a mathematical model for predicting the dynamics of bacterial spore populations affected by activation and inactivation when subjected to lethal temperature. This model differs from a recently proposed model by the hypothesis that inactivations of dormant and activated spores are different rather than identical and contribute to accurate predictions of actual survivor curves. Inclusion of activation in the model represents the basis for any increase of the survivor population early in a thermal process before its long decline due to predominant inactivation. In addition, the presence of activation precludes the need for conventional heat shock to obtain a homogeneous population of activated spores prior to testing a thermal process.

Analytical solution of the model with the initial conditions provided the response equation for activated spores that are the survivors of a heat treatment. Isothermal experiments using spores of Bacillus stearothermophilus in capillary tubes were performed to obtain three independent sets of survivor curves for each temperature at 105, 110, 115, and 120°C.

Rate constants for the model and their variations with temperature were obtained by regression analysis of survivor data. Responses of four models, including the new model and the conventional, single-term model, were compared and showed the superiority of the new model. The model adequately described isothermal survivor curves at the four constant temperatures, and the temperature dependencies of its rate constants were well described by the Arrhenius equation. Survivor curves predicted by the model, using Arrhenius equations for rate constant variations, showed close agreement with experimental data when spores were subjected to non-isothermal, dynamic heating processes in the sub-UHT (100-120°C) range. Extrapolation of the rate constants to the UHT (125-150°C) range by the Arrhenius equations established in the sub-UHT range permitted the model to predict adequately the final numbers of survivors of dynamic heating processes in the UHT range as well. Shortcomings of the traditional single term model were clearly indicated and revealed the importance of the new model to dependable design of sterilization processes in the UHT range.

CHAPTER 1

INTRODUCTION

1.1 Background

Analytical and numerical models that predict dynamics of bacterial spore populations during sterilization processes are based only upon an assumed first order inactivation transformation of heat shocked (activated) spores. Response of these models often differ from experimental data. These discrepancies are more pronounced during validation of ultra high temperature (UHT) sterilization processes operating between 125 to 150°C. Because actual kinetic data is difficult to generate at these temperatures, largely due to the requirement of sophisticated equipment, process design at these higher temperatures is generally based upon extrapolation of thermal death time (TDT) data obtained at lower temperatures. However, experiments performed in this range have revealed the shortcomings of these extrapolations. Even use of the Arrhenius equation in extrapolating to the UHT range has not provided accurate predictions.

To overcome these limitations of the conventional single term model, more descriptive models that include activation have been proposed. Shull et al. (1963) assumed that a significant fraction of the original population (direct microscopic count) was in the dormant state and developed a mathematical model to account for the characteristic shoulder of survivor curves by attributing it to heat activation. They postulated that the transition from dormancy to the activated state and, finally, to the dead state was sequential and could be represented by two first order differential equations. Rodriguez (1987) proposed a model that assumed concurrent and independent

activation and inactivation transformations. Tested with spores of Bacillus subtilis, the Rodriguez model showed reasonably good agreement between experimental and predicted survivors. In developing that model, the authors assumed the transformation inactivating dormant spores was the same as that for activated spores. However, activation induces many changes in a spore suspension. It increases the germination rate, activates enzymes which are dormant in resting spores, changes the requirements for induction of germination, and, possibly, results in reversible changes in the tertiary structure of one or more proteins controlling the dormant state of a spore. It is, therefore, prudent to hypothesize that the transformation inactivating viable dormant spores is different from that of activated spores.

It was therefore important to develop a mathematical model that accounted for concurrent transformations of activation of dormant spores, and, distinct inactivation of dormant and activated spores. It was equally important to test the new model for application to thermophilic spores of B. stearothermophilus, which often are used as biological indicators to monitor efficacy of sterilization processes because of their high heat resistance, non-pathogenicity, and ease of culture. They also cause flat-sour spoilage in low acid foods.

1.2 Objectives

The work reported in this dissertation was designed to overcome the above past deficiencies and omissions by addressing the following specific objectives:

i) Develop and test a mathematical model consisting of concurrent transformations accounting for activation of dormant spores and distinct inactivations of dormant and activated spores and applicable to prediction of population dynamics of thermophilic bacterial spores subjected to lethal temperature.

ii) Conduct isothermal experiments with dormant B. stearothermophilus spores providing survivor curves at several temperatures in the sub-UHT range (100-120°C) from which rate constants of the model could be obtained.

iii) Develop procedures for estimating rate constants of the model from survivor curves at constant temperature.

iv) Determine the effect of temperature on each rate constant in the model.

v) Validate the model in a dynamic situation by subjecting spores to non-isothermal temperature histories in the sub-UHT range.

vi) Validate application of the model to UHT ranges, using extrapolation of rate constant values calculated from survivor curves generated at lower temperatures.

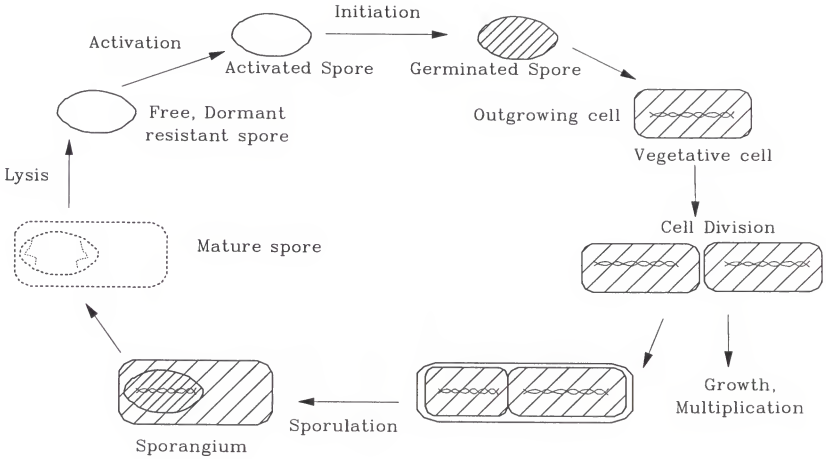
CHAPTER 2

LITERATURE REVIEW

Nutritional deprivation causes a normal bacterial cell to stop replicating by cell division and, instead, to initiate a new set of biochemical and morphological changes that eventually give rise to a spore in a process called sporogenesis. Fig. 2.1 is a diagrammatic representation of the cycle of bacterial spore formation, germination and outgrowth. Bacterial spores are far more resistant to heat than are vegetative forms of bacteria, and, thus, are of primary concern in the sterilization of most foods. All species of spore-formers are in the family Bacillaceae, and most recognized Bacillaceae species of concern to food processors are members of the aerobic genus Bacillus and the anaerobic genus Clostridium. They include many important pathogenic and spoilage bacteria, and they determine the requirements for sterilization and preservation of many foods. In this chapter, relevant aspects of bacterial spore formation, reverse transformation into a vegetative cell, morphology, and maintenance of heat resistance are discussed. This chapter also discusses thermal inactivation and activation of bacterial spores at high temperatures, and the nonlinear behavior of survivor curves. Finally, methods for measuring heat resistance of bacterial spores are described.

2.1 Sporulation

Sporulation starts when a living, vegetative cell senses nutritional depletion and causes it to cease normal cell division and instead form polar septa that partition the sporulating cells into mother-cell and forespore compartments. This asymmetric septation usually depends on a nutritional signal or trigger and the subsequent



2.1 Cycle of spore formation, activation, germination, and outgrowth (Gould, 1984)

forespore development depends on the genetic constitution of the organism (Freese, 1981). General characteristics of sporulation appear to be common to all bacterial spore-formers. The extent and rate of sporulation is strain dependent and affected by environmental conditions. The type of nutrient limitation also affects the degree of spore heat resistance. Sporulation is not a form of microbial reproduction, but transformation of the organism into an alternative form, and usually only one spore is produced from one vegetative cell.

2.1.1 Initiation

The actual mechanism initiating sporulation of a vegetative cell is unknown, but maximum capability for sporulation coincides with the completion of DNA replication during the normal growth/reproduction cycle of a bacterium. Depending upon the triggering mechanism and whether the cell decides to sporulate, it is not irreversibly committed to becoming a spore until later in the process. Commitment is nutritionally dependent; cells grown in a minimal medium achieve commitment earlier than the cells grown in a rich medium. However, if a cell decides to replicate, it cannot sporulate until replication is completed.

The relevance of specific nucleotides or their synthesis to the initiation of sporulation has been widely studied. Levels of Guanosine diphosphate (GDP) and Guanosine triphosphate (GTP) are consistently lower early in sporulation than during vegetative growth (Lopez et al., 1981). Freese (1981) proposed that nutrient limitation leading to low guanine nucleotide level may trigger sporulation. It is not known how low levels of GDP and GTP are induced in the cell, and, even if low levels of GDP and GTP trigger sporulation, the mechanism is not known. The highly phosphorylated nucleotide, adenosine-bis-triphosphate (Abt), has been correlated most successfully with the onset of sporulation and apparently regulates sporulation at the transcription level (Rhaese et al., 1978). Absence of Abt synthesis causes a lack of sporulation-type

ribosomes essential for sporulation. Roles of GTP and Abt have not been explored but their involvement is clearly one of the earliest sporulation events. It is evident that many factors of the growth cycle have been implicated in the initiation of sporulation; however, the exact mechanism still remains elusive and speculative.

2.1.2 Sequence of events

To correlate biochemical and morphological changes occurring in sporulating cells, it is important to synchronize the populations. This is achieved to a great degree by manipulating environmental conditions. Mutants blocked at various stages in sporulation have also been used to determine the sequence of sporulation events (Gombas, 1987). Sporulation has been divided into seven recognized stages. Fig. 2.2 charts the sequence of these changes.

Stage 0

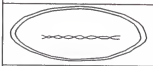
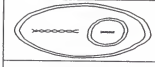
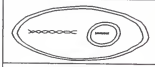
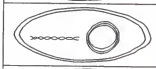
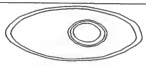
Cells in this stage are morphologically similar to those of vegetative cells, and during this stage or the next, the genome is replicated and events of initiation take place.

Stage 1

This stage is characterized by the alignment of chromatin from a spherical coil to a single axial filament. Mesosomes, which are tubular involutions of the cytoplasmic membrane often associated with cell division, occur at 10 to 17% of the cell length from either one or both poles, even in the absence of protein synthesis.

Stage II

Starting at one of the subpolar mesosomes, invagination of the cytoplasmic membrane separates part of the cell and at least one copy of the genome from the rest of cytoplasm by a double membrane. Little cell wall material is deposited between the walls of the membrane, and sporulation-type ribosomes first appear during this stage (Fortnagel and Freese, 1977). Spore-specific protein synthesis is initiated in this or the next stage.

	Stage 0 Vegetative form, start of differentiation
	Stage I Chromatin filament formed, proteases excreted
	Stage II Spore septum formed, copy of genome isolated
	Stage III Protoplast engulfed, cell committed to sporulation
	Stage IV Cortex synthesized, Calcium and DPA incorporated, refractile spore
	Stage V Spore Coats formed, Resistant to Octanol
	Stage VI Maturation, Resistant to heat
	Stage VII Autolysis, Release from sporangium

2.2 Stages in bacterial spore formation (Gould and Dring, 1974)

Stage III

The forespore is engulfed when the invagination point at the cell wall moves toward the polar end of a cell. The size of the septum double membrane also increases in this stage. Commitment is nutritionally dependent. Cells grown in a minimal medium achieve commitment at stage II, while cells grown in a rich medium become committed only after completion of engulfment, and at this stage addition of nutritional supplements will not reverse the sporulation process. Exosporium precursor in Bacillus cereus is also detected at this time, implying spore-specific protein synthesis (DesRosier and Lara, 1984).

Stage IV

Cell wall synthesis occurs between the double walls. Cortex subunits are synthesized in the sporangium and assembled by the outer forespore membrane. Subunits of germ cell wall are synthesized in the forespore and assembled by the inner membrane. During this stage, a spore becomes refractile and begins DPA synthesis and uptake of calcium into the forespore. Calcium moves into the sporangium by active transport and into the forespore by facilitated diffusion, using calcium specific carrier (Hogarth and Ellar, 1978). Inside the forespore calcium is presumed to chelate with dipicolinic acid (CaDPA), thereby decreasing its concentration and allowing facilitated diffusion to continue. DPA is synthesized in the mother cell and transported into the forespore. The protoplast shrinks before cortex synthesis, and subsequent shrinkage may be facilitated by the cortex.

Stage V

Deposition of spore coat material is initiated during this stage, and cortex synthesis and accumulation are completed. The extracted forespore is osmotically stable, and resistance to octanol, hydrogen peroxide, and enzymes is acquired in this stage.

Stage VI

Heat resistance, spore maturation, and attributes of dormancy are acquired during this stage.

Stage VII

Autolytic enzymes lyse the cell wall and release the spore. The spore is mature, and will stay in a dormant state, until conditions for germination occur.

2.2 Germination

Germination broadly refers to transition of a dormant spore into a vegetative cell, reversing the process initiated by sporulation. It is the process by which a spore, in response to appropriate environmental stimuli, leaves the dormant state, undergoes rather drastic morphological and biochemical changes, and eventually synthesizes substances and structures which permit resumption of vegetative existence. This process involves three distinguishable stages: activation, germination (also referred to as triggering and initiation), and outgrowth (Keynan and Halvorson, 1965).

2.2.1 Activation

Activation changes viable spores incapable of germinating in a growth medium into activated spores capable of germination and growth. Activation of most bacterial spores is considered to be a reversible phenomenon probably related to macromolecular reconfiguration (Handley, 1977). Spores can be activated by various treatments, including ionizing radiation, reducing agents, oxidizing agents, extremes of pH, sonication, high pressure, and sub-lethal heat. These treatments are consistent with the hypothesis that activation results in structural rearrangement that may increase permeability to germinants. Sub-lethal heat treatment appears to break hydrogen bonds, thereby creating some molecular mobility. However, on storage at low temperature, broken bonds apparently reform, probably as a result of high concentrations of

the constituents and hence close proximity of the broken bonds (Lewis et al., 1965; Keynan and Evenchik, 1969). A heat activated spore does not lose its characteristic spore properties, but its requirements for the induction of germination are different.

2.2.2 Germination

Germination is the irreversible loss of spore properties that can be triggered by a specific group of low-molecular weight compounds. These include sugars (glucose, fructose), amino acids (alanine, leucine, proline), purines (adenosine), salts (calcium-DPA, KBr), detergents (dodecylamine), and high pressure. A germinated cell is metabolically active, heat-labile, nonrefractile, stainable; yet it is distinct from a vegetative bacterium. Germination involves triggering and initiation. Triggering is the interaction of a spore with a germinant that irreversibly commits it to the degradative phase that follows. Since triggered spores do not require continued presence of a germinant, it is presumed that it binds to a receptor molecule (possibly in the inner forespore membrane) and triggers a biophysical change resulting in the early events of germination (Setlow, 1981).

Initiation is the degradative phase that follows triggering. Various cortex-lytic enzymes and proteases are activated and cause hydrolysis of cortex, coat, and several core and membrane proteins. The spore loses its characteristic properties, including rigid structure, refractility, absence of metabolic activity, resistance to physical and chemical challenges and release of calcium and DPA (Lefebvre and Leblanc, 1988).

2.2.3 Outgrowth

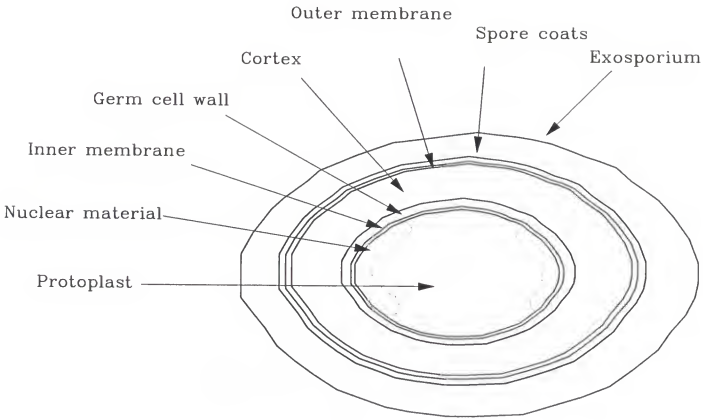
Outgrowth refers to the final transition of a spore into a vegetative cell by biosynthesis and repair of new macromolecules, characteristic of a vegetative cell. Morphologically, outgrowth involves swelling of the protoplast, rupture of the remaining spore cortex, coats and exosporium, and emergence of the new cell. Conditions

for outgrowth differ from those supporting germination, including different optimum outgrowth temperature and need for exogenous nutrients to complete the outgrowth process. Outgrowth is generally more susceptible to inhibition than germination. Syntheses of proteins, RNA, membrane, and cell wall are initiated soon after germination, along with transcription, translation, and various other processes of vegetative cells. Replication of DNA occurs late in outgrowth. This permits repair of DNA injury during dormancy and germination, before replication (Ginsberg and Keynan, 1978; Lammi and Vary, 1972).

2.3 Morphology of Bacterial Spores

A spore is generally a spheroid with several concentric layers (Fig. 2.3). Spores of all species have the same basic structure, a central protoplast, surrounded in turn by a plasma membrane, germ cell wall, cortex, spore coats and exosporium. The cortex and germ cell wall are specialized adaptations of vegetative structures while spore coats, exosporia, and appendages are spore specific structures.

The vital part of a spore, the small protoplast, contains all components essential for life of the cell, including genetic material, ribosomes and enzymes necessary for outgrowth; it is packaged within a complex of 6 to 7 cytologically and chemically distinct layers. A spore also contains unique substances such as dipicolinic acid, DPA (pyridine-2,6-dicarboxylic acid) and high concentrations of calcium. A spore is metabolically inert, has great longevity and extreme resistance to heat, mechanical stress, desiccation, high hydrostatic pressure or vacuum, electrical forces, irradiation, freezing, antibiotics, disinfectants, organic solvents and many chemicals. A spore is also resistant to enzymes external to the protoplast, DNA repair, and other repair mechanisms that may operate during germination (Murrell, 1988).



2.3 Cross-section of a bacterial spore (Algie, 1983)

Exosporium and appendages

The outermost layer of a spore is composed of an exosporium or filamentous appendages. Exosporium is present in many Bacillus and Clostridium spores but is not produced by all sporeformers, and its presence is strain dependent. Appendages are loosely fitting structures commonly found on spores of Clostridium species. It is believed that exosporium and appendages have little significance to heat resistance of spores. In some species, the exosporium is a loose sac about a mature spore while it is tightly fitting or even integral with the spore coats of other species (Sousa et al., 1976). In B. cereus, Matz et al., (1970) showed that the exosporium was loosely fitted and could be selectively removed by mechanical shearing. Their chemical analysis suggested a composition predominantly of proteins, carbohydrates, and lipids. Tipper and Gauthier (1972) suggested that the exosporium may prevent assembly of coat subunits elsewhere in the cell during spore formation. However, since exosporium and appendages form the outermost layer of a spore, they determine properties, such as adhesion and antigenicity, of obvious ecological importance.

Spore coats

Spore coats are the outer layers with any real significance to spore properties. They comprise more than 50% of a spore and consist predominantly of structural proteins. X-ray analyses have revealed periodic crystalline structures resembling α-keratin and β-keratin (Kadota et al., 1965a, 1965b). Three distinct layers form the spore coats, an electron dense, cross-patched outer layer, an electron transparent, pitted middle layer, and an electron dense innermost layer. The innermost layer is believed to originate from physical entrapment of the mother cell cytoplasm between the middle layer and the outer forespore membrane; such entrapment would suggest similarity of inner layer and mother cell cytoplasm compositions relative to ribosomes and various enzymes (Ohye and Murrell, 1973). It has also been speculated

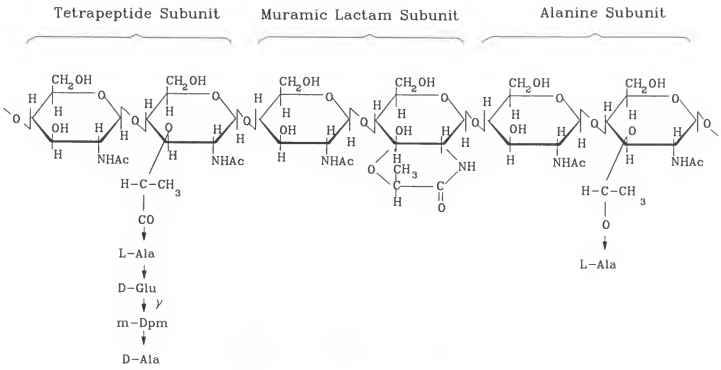
that these enzymes, if they remain active, may be involved in germination. Extraction or removal of spore coats does effect germination (Aronson and Fitz-James, 1976).

Spore coats are believed to have little influence on heat resistance of a spore. Spores whose coat structure may be disrupted by inhibition of synthesis or extraction of coat proteins retain their calcium and DPA contents and refractility, and they are generally heat and radiation resistant. However, these spores are sensitive to lysozyme and octanol and may show differences in their responses to germinants, all of which suggests that spore coats provide a protective permeability barrier preventing access by a wide range of deleterious substances, particularly enzymes and surfactants, to the sensitive cortical peptidoglycan and other interior parts of the spore.

Cortex

The cortex, comprising about 30% of the spore volume, is a thick (50-100 nm) laminated layer surrounding the germ cell wall. In mature spores it appears as an electron transparent zone between the core and the coats. Upon germination, the cortex loses its refractiveness to staining and a fibrous network can be observed.

The cortex is largely a 3-dimensional, peptidoglycan-polymer matrix made up of repeated units of the amino-sugars N-acetyl glucosamine and muramic acid lightly cross-linked by the tetrapeptide side-chain comprised of L-alanine-D-glutamic acid-m-diaminopimelic acid and D-alanine (Fig. 2.4). These units are not randomly arranged, and alanine and tetrapeptide units are rarely contiguous but are most frequently separated by a single muramic lactam subunit. Cortical peptidoglycan differs from that of gram-positive vegetative cell to the extent that about 45-60% of the muramic acid residues in the cell wall glycan polymer lack both a peptide and an N-acetyl substituent and instead form an internal amide "muramic lactam" (Warth, 1978).



2.4 Peptidoglycan units from Bacillus subtilis spores (Warth, 1978)

Biophysical studies suggest cortical peptidoglycan is a highly elastic, flexible polymer well designed for a role in containing the hydrostatic pressure of the low molecular weight, solute-packed spore protoplast.

The cortex plays a significant role in the maintenance of heat resistance and germination of a spore (Murrell, 1988). Imae and Strominger (1976) controlled the extent of cortex formation in Bacillus sphaericus spores by limiting the supply of exogenous meso-diaminopimelic acid (Dpm) to the mutants defective in Dpm synthesis. They showed that about 10-20% cortex was needed to obtain refractility and DPA accumulation, slightly more for xylene and octanol resistance, but about 90% was required for heat resistance. Resistance may also require accumulation of Ca and DPA in the spore. Balassa et al. (1979) showed in DPA negative mutants of B subtilis spores that heat resistance was achieved only in presence of external DPA.

Outer and inner spore membranes

The outer spore membrane, between spore coats and cortex, and the inner spore membrane, between germ cell wall and protoplast, both originate from the mother cell cytoplasmic membrane but differ in lipid and protein compositions (Swerdlow and Setlow, 1984; Crafts-Lightly and Ellar, 1980). These membranes provide permeability barriers to small molecules such as glucose, calcium and DPA (Koshikawa et al., 1984). Protoplasm membrane isolated from Bacillus megaterium differs in composition from plasma membrane of a vegetative cell. It contains 66% of its lipid and 80% of its phospholipid, a unique red carotenoid pigment, and a membrane bound electron transport cytochrome system (Murrell, 1988).

Germ cell wall

The germ cell wall is the first layer outside the plasma membrane, and it develops into normal vegetative cell wall during outgrowth. Morphology of the germ cell wall suggests that it is formed earlier than

the cortex and synthesized by the forespore cytoplasm and membrane, whilst the cortex is synthesized by the mother-cell cytoplasm and outer forespore membrane. Its structure is hypothesized to be a rudimentary form of cell wall lacking some of the distinctive features of a vegetative cell wall, but it probably does not have the unique structural features of cortical peptidoglycan. It is resistant to lysozyme and lytic enzymes when lytic digestion of the cortex occurs during germination.

Protoplast

The protoplast is the core of a spore and the location of all essential components for life of the cell - genetic material, ribosomes, membrane elements or complexes, and enzymes necessary for outgrowth. Macromolecular constituents of the core generally are very similar to those of a vegetative cell, however, the compositions of smaller molecules of a spore differ dramatically from those of a vegetative cell. The protoplasm contains free solutes, including 3-phospho-D-glyceric acid, glutamate, basic proteins, etc, and high concentrations of DPA (20-30% on dry weight basis) and calcium (6-8% on d.w.b) (Murrell, 1988). Location of most of the DPA, Calcium and other elements (Mg, Mn, P) in the protoplast (Stewart et al., 1980) is believed to affect significantly the biophysical state of the protoplast and the resistance of a spore.

2.4 Heat Resistance of Bacterial Spores

The resistance of bacterial spores to heat varies greatly. In many Bacillus species, it correlates well with the maximum temperature for growth of the vegetative form. A spore has about 40-50°C higher inactivation temperature than a vegetative cell. Heat resistance between species or even individual spores varies greatly. For example, 90% of a C. botulinum (Types A and B) spore population is inactivated in less than 0.2 second at 121.1°C while spores of B. stearothermophilus

take about 4 to 5 minutes at the same temperature (Stumbo, 1965). Even after a spore is fully formed, heat resistance can be affected by multiple factors. Gombas (1983), Lindsay et. al (1985), Murrell (1988), and Gerhardt (1989) presented comprehensive papers examining various theories on development and maintenance of the heat-resistant state of bacterial spores. Spore survival at high temperature depends upon stabilization of all vital, potentially labile components. Although individual components may have specific adaptations, a general mechanism for stability is required. Heat resistance theories can be classified into two major groups.

2.4.1 Heat resistance due to partial dehydration of the protoplast

Murrell and Scott (1966) showed in spore species with widely differing moist-heat resistances a significant increase in resistance as their a_w decreased, with maximum resistance occurring for a_w between 0.2 and 0.4. However, their study did not consider the distribution of water within a spore. The interior of a spore being highly refractile indicates a state of partial dehydration. Proteins, the most important class of heat labile components, show consistently increased heat stability upon drying. Theories on how the spore protoplast becomes dehydrated during sporogenesis have focused on mechanical processes in the cortex wherein the protoplast is dehydrated and held in this state by

i) a contractile cortex,

ii) an expanded osmoregulatory cortex,

iii) reverse osmosis during cortex formation and

iv) an anisotropically swollen cortex.

The contractile cortex theory maintains that the cortex holds the protoplast in a dehydrated state by mechanical pressure. Although there is no direct evidence to substantiate this theory, cross linking of glycan chains could bear this tension.

According to the expanded osmoregulatory cortex theory (Gould and Dring, 1975a, 1975b), electrostatic repulsion of free carboxyl groups in

the cortical polymer causes the cortex to expand against the spore coats, thereby applying pressure to the protoplast. They also proposed that an osmotically active cortex would take up water, causing the cortex to swell and partially dehydrate the protoplast. This theory is supported by the absence of sufficient peptidoglycan to fill the cortical space, adequate coat strength, and inward expansion of the cortex in electron micrographs of disrupted spores. However, coat-less mutants have retained their heat resistance (Cassier and Ryter, 1971) and the biophysical state of a forespore is developed before cortex and coat formation is complete (Murrell, 1981).

Reverse osmosis (Algie, 1980) seems to be an unlikely basic mechanism for major stabilization, although it might be operating at some stage of the spore development (Murrell, 1988).

Warth (1985) suggested the anisotropically swollen cortex theory. He found that a_w=0.73 was required to stabilize proteins within the protoplast against heat. That would require turgor pressure ≥ 52 MPa within the spore, which would have to be sustained by cortical tension. He believed the cortex was in tension in its concentric layers but in compression radially. Moreover, CaDPA uptake would displace additional water and increase the turgor pressure.

However, major changes in the protoplast and its volume occur before substantial cortex and coat synthesis occurs (Murrell, 1981). Permeability studies with labelled water indicate that water molecules permeate all parts of the spore (Black and Gerhardt, 1962). Studies using nuclear magnetic resonance (Bradbury et al., 1981) and sorption isotherms (Watt, 1981) indicate water content of the core equal or greater than that of the cortex. However, these procedures involve extrapolation of results from isolated spore fractions to intact spores. <u>In situ</u> measurements of water content by Beaman et al. (1984) within fully hydrated, dormant spores, with defective coat and outer membrane complex, indicated lower water content of protoplast than the cortex.

2.4.2 Heat resistance due to general molecular stabilization

According to this theory, heat stability depends upon molecular rearrangements within the protoplast. A considerable number of biochemical changes occur during stage IV of sporulation, which precedes cortex synthesis and DPA formation. Cellular DNA changes from an electron-transparent to a more fibrous appearance, and it moves to the periphery of the forespore. The protoplast volume is reduced by about 50%, which is believed to occur as a result of plasmolysis, germ cell wall contraction, or synaeresis. Plasmolysis involves redistribution of cations in the protoplast and the surrounding space, and synaeresis involves molecular reordering with the exclusion of water (Murrell, 1981).

At this time, there is a significant accumulation of solutes, perhaps increased hydrophobicity, and reduction of molecular mobility of DNA, all of which impose severe constraints on molecular and macromolecular motions leading to heat denaturation (Murrell, 1981). Setlow et al. (1982) indicated that low molecular weight basic proteins accumulating at this time might bind to DNA and affect its thermal stability. Calcium moves into the sporangium by active transport and into the forespore by facilitated diffusion, using calcium specific carrier (Hogarth and Ellar, 1978). Inside the forespore calcium is presumed to chelate with dipicolinic acid (CaDPA), thereby decreasing its concentration and allowing facilitated diffusion to continue. Calcium accumulates before DPA and may not be bound to DPA initially. Grecz et al. (1972) suggested that DPA and calcium are chelated in the spore with amino acids and peptides, resulting in heat stability. Lindsay et al. (1985) were of the opinion that from stage IV on, calcium and DPA specifically bind with available nucleic acids and stabilize them to heat. Lindsay and Murrell (1986) examined the effect of DPA and CaDPA on the spectral characteristics of nucleic acids. Their analysis indicated that both DPA and CaDPA bind to various RNA species, although

the mechanism of binding was not elucidated. They concluded that water was removed from around the molecules of spore polynucleotides and out of the protoplast by their hydrophobic reaction with DPA and CaDPA. While the protoplast is in the contracted state, the cortex is formed about the protoplast in a stable cross-linked state which eliminates the need for large cortical contraction pressure on the protoplast to reduce its volume. The coats are finally completed around the cortex, protecting it from lytic enzymes released by lysing mother cells. The above changes are accompanied by sequential increases in chemical and heat resistances. They further suggested that essential heat labile macromolecules bond with calcium dipicolinate by electrostatic, and hydrogen bonds and are heat stabilized.

The two theories are not mutually exclusive. Reduction in water could increase interactions between spore solutes, resulting in specific stabilization in addition to general stabilization due to dehydration. "Factors involved in the development and maintenance of the heat-resistant state are complex and, although there may be one mechanism more important in achieving this state, all the mechanisms such as enzyme stabilization, chelation, stable gel formation, contraction or expansion of the cortical polymer, and osmo-regulation may be well be involved to some extent. To prove conclusively, experimentally which mechanism is the most important may be very difficult task. Certainly, however, no theory to date is fully acceptable as a basic resistance mechanism" (quoted in Murrell, 1981, p 64).

2.5 Thermal Inactivation of Bacterial Spores

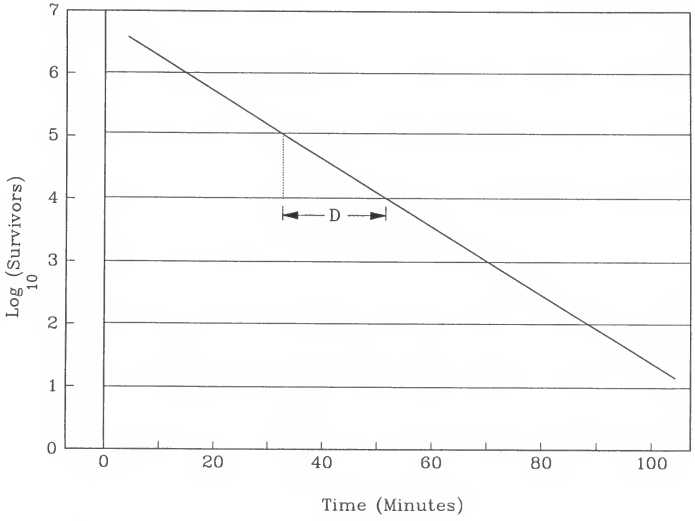
Thermal inactivation of a bacterial spore is achieved when the spore loses its ability to germinate and reproduce. From the earliest quantitative measurements by Chick (1910) with hot water and *Bacillus coli commune* through Esty and Meyer's (1922) working with *C. Botulinum* to the present, number of spores generally reduces exponentially with

time of exposure to lethal temperature. If the logarithm of number of survivors is plotted against time of exposure, a straight line is obtained (Fig. 2.5) which is commonly referred to as the logarithmic order of death. The presence of a shoulder in the initial portion of the semi-log plot of a survivor curve, often attributed to activation of dormant spores, has been dealt with by either heat activating the spores prior to lethal heat treatment or neglecting this portion of the curve in analyses of survivor data. The activation phenomenon and the deviation of survivor curves from logarithmic behavior is dealt with later in this chapter (Sections 2.6 and 2.7). However, in this section, literature applicable to the exponential portion of a survivor curve is discussed. This exponential response is similar to that of a unimolecular or first-order, bimolecular, chemical reaction. In unimolecular reactions, only one substance reacts, with a rate of reaction directly proportional to its concentration; in a first order, bimolecular, reaction, one reactant is in such great excess that variation in its concentration is negligible, and the rate of decomposition of the second reactant is directly proportional to its concentration (Stumbo, 1965). This behavior led Rahn (1945) to reason that death of a bacterial cell must be due to denaturation of a single molecule essential to reproduction.

In a single species suspension of spores at constant temperature, the rate of thermal inactivation of the spores can be expressed by a first-order differential equation in which the rate is proportional to the number of spores,

$$\frac{dN}{dt} = -K N \qquad (2.1)$$

where N is the concentration of viable spores at a given time and K is the inactivation rate constant in min^{-1}.



2.5 Semi-log plot of survivor curve

Rearranging Eq. (2.1) and integrating we get

$$\int_{N_0}^{N_t} \frac{dN}{N} = -\int_{t=0}^{t} K\, dt \tag{2.2}$$

or

$$\ln \frac{N_0}{N} = K\, t \tag{2.3}$$

where N_0 is the initial number of spores at t=0 and N is the number at time t.

Eq. (2.3) can be written as

$$N(t) = N_0\, e^{-K\, t} \tag{2.4}$$

In food processing, microbiologists often use the decimal reduction time D, defined as the time required to inactivate 90% of the spores at constant temperature (Fig. 2.5), to characterize a sterilization process. Solving Eq. (2.4) we get,

$$\frac{N(t)}{N_0} = \frac{1}{10} = e^{-K\, D} \tag{2.5}$$

Taking the $\log_{10}$ of both sides and solving for D,

$$D = \frac{2.303}{K} \tag{2.6}$$

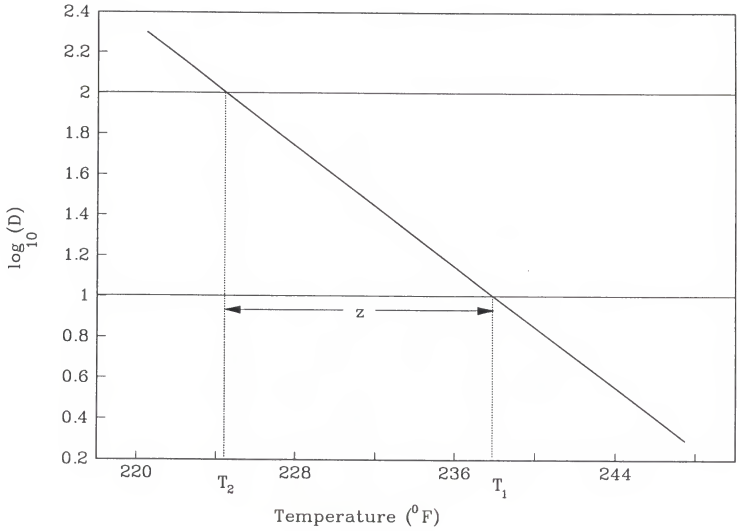
Combining Eqs. (2.3) and (2.6),

$$\log_{10} \frac{N_0}{N(t)} = \frac{t}{D} \tag{2.7}$$

Temperature dependency of inactivation rate constants has been commonly expressed by the TDT curve, Arrhenius equation, and the Q_{10} theory.

2.5.1 Thermal death time curve

A thermal death time (TDT) curve is the semi-log plot of decimal reduction time D versus temperature, as in Fig. (2.6), where parameter z is the temperature interval, in degrees Fahrenheit, required for the TDT



2.6 Thermal death time (TDT) curve

curve to traverse one log cycle. The parameter z is employed in process characterizations to express relative resistance of spores to different temperatures. Since the plot is a straight line, the equation of a TDT curve can be expressed as

$$\log_{10} D_{T_2} - \log_{10} D_{T_1} = \frac{1}{z}(T_1 - T_2) \tag{2.8}$$

Lethality F, is defined as the time required for specified reduction of the population of viable spores. From Eq. (2.7), lethality at constant temperature is expressed by

$$F = D \log_{10}\left(\frac{N_0}{N}\right) \tag{2.9}$$

Since D varies with temperature, lethality F is conventionally expressed relative to the reference temperature, 121.1°C (250°F), and

$$F_0 = D_{121.1} \log_{10} \frac{N_0}{N} \tag{2.10}$$

is the lethality of a process in minutes at 121.1°C. For the reference temperature of 121.1°C, Eq. (2.8) can be written as

$$D_T = D_{121.1} 10^{\frac{(121.1-T)}{z}} \tag{2.11}$$

Combining Eqs. (2.10) and (2.11) with Eq. (2.7), we get the F_0 of a process a any temperature T

$$F_0 = t\, 10^{\frac{(T-121.1)}{z}} \tag{2.12}$$

Eq. (2.12) permits comparison with lethality at 121.1°C for any time-temperature combination. To calculate lethality of a sterilization process with a variable temperature regime, Eq. (2.12) can be integrated numerically for several different temperature stages T_1, T_2, and so on, each having different times t_1 t_2,...; the F_0 values at each stage are

added to give the total F_0.

$$F_0 = t_1 10^{\frac{T_1-121.1}{z}} + t_2 10^{\frac{T_2-121.1}{z}} + \ldots\ldots \quad (2.13)$$

Linear dependence of the inactivation rate constant on temperature was also shown by Townsend et al. (1938), Anderson et al., (1949), and Tischer and Hurwicz (1954). However, all those findings were restricted to narrow ranges of temperature, as was pointed out by other workers (Rahn, 1945; Levine, 1956) who cautioned that extrapolation of those results to wider temperature ranges could lead to serious errors, since linear dependence of inactivation rate constant on temperature may not hold at higher temperature. Deviation of TDT curves from linearity was also shown by Pflug and Esselen (1953), Jones (1968), and Jonsson et al. (1977).

2.5.2 Arrhenius equation

To account for the effect of temperature on the rate of glucose hydrolysis, Arrhenius first proposed the equation:

$$K = Ae^{-\frac{E_a}{RT}} \quad (2.14)$$

where K = rate constant ($time^{-1}$), A = frequency constant ($time^{-1}$), E_a = activation energy (Kcal/mol), T = absolute temperature (°K), and R = gas constant (1.9872×10^{-3} Kcal/mol°K). Although the Arrhenius equation was developed empirically, it has been used successfully to describe the effect of temperature on inactivation rate constants of bacterial spores over wide temperature ranges.

Eq. (2.14) can also be written as

$$\ln(K) = A + (-\frac{E_a}{R})\frac{1}{T} \quad (2.15)$$

and A and E_a can be obtained, respectively, from the intercept and slope of a plot of the natural logarithm of inactivation rate constants vs. reciprocal absolute temperature. Substituting Eq. (2.14) into (2.2) yields

$$\ln\frac{N_0}{N(t)} = A\int_{t=0}^{t} e^{-\frac{E_a}{RT}}dt \qquad (2.16)$$

which can be used in the design of sterilization processes for variable temperature regimes.

Wang et al. (1964) studied kinetics of the death of Bacillus stearothermophilus spores using a tubular flow reactor. Death rate constants obtained from survivor curves at different temperatures were found to be described well with the Arrhenius equation whereas the TDT plot did not yield a straight line. By combining Eq. (2.6) and (2.14), it can be shown that the plot of $\log_{10}D_T$ vs. 1/T (T in °K) is a straight line and the TDT curve, which is a plot of $\log_{10}D_T$ vs. T can be approximated by a straight line only over a small temperature range. Perkin et al. (1977) showed thermal death rates of B. stearothermophilus spores could be related to temperature by the Arrhenius equation up to 150°C. However, David and Merson (1990) concluded that use of the Arrhenius equation did not provide more accurate predictions than the conventional TDT relationship. Working with spores of B. stearothermophilus, they obtained inactivation rate constants with a computer controlled reactor. Since their TDT curve was nonlinear in the high temperature range, they described the data by two straight line segments with different z values. An Arrhenius plot did not represent the data better than the two TDT curves.

2.5.3 Q_{10} theory

Rahn (1945) put forward the Q_{10} theory for temperature dependency of rate constants. Parameter Q is defined as the quotient of rate constants and indicates how much more rapidly death proceeds at temperature T_2 than it does at lower temperature, T_1.

Since from Eq (2.3)

$$K_1t_1 = K_2t_2 \qquad (= \ln(\frac{N_0}{N(t)})) \qquad (2.17a)$$

$$Q = \frac{K_2}{K_1} = \frac{t_1}{t_2} \tag{2.17b}$$

where K_1 and K_2 are the inactivation rate constants at T_1 and T_2 respectively, and t_1 and t_2 are the corresponding inactivation times. For comparative purposes, it is common to define the coefficient for an increase of 10°C and is designated as Q_{10}.

$$Q_{10} = \frac{Rate\ at\ (T+10)}{Rate\ at\ T} = \frac{K_{T+10}}{K_T} \tag{2.18}$$

Rahn showed that value of z for the TDT curve relates to Q_{10} as follows:

$$z = \frac{18}{\log_{10} Q_{10}} \tag{2.19}$$

2.6 Thermal Activation of Bacterial Spores

Activation changes viable spores incapable of germinating in a growth medium into activated spores capable of germination and growth. Sub-lethal heat is most often used in the laboratory for activating spores. Curran and Evans (1945) were the first to demonstrate systematically that sublethal heat would induce dormant spores to germinate. Finley and Fields (1961) demonstrated with B. stearothermophilus spores that true activation could occur above 100°C, with the degree of activation being strain dependent and variable among spore suspensions of the same strain. A relationship was evident between the period of time and the temperature of a heat treatment used to produce activation. Busta (1963) systematically evaluated time-temperature relationships of heat activation as measured by colony counts. He used spores of B. subtilis and activated them at eight temperatures ranging from 5 to 94°C. He concluded from the kinetic data that activation appeared to occur throughout the entire range of temperatures and was a function of time. Busta also demonstrated that velocities of activation reaction at different temperatures could be

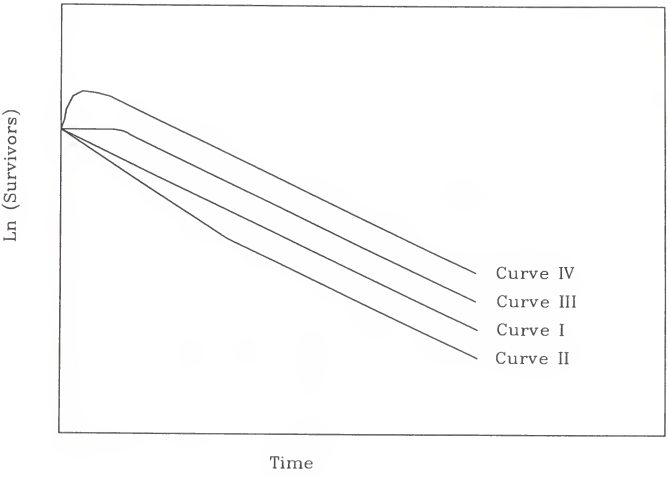
described adequately by the Arrhenius equation. Levinson and Feeherry (1978) measured activation by percent increase in i) colony forming units and ii) germination rate in spores of Clostridium perfringens.

Brown et al. (1968) indicated that without prior heat activation less than 10% (of chamber count) of spores of B. stearothermophilus germinate and produce colonies on a nutrient agar medium. Optimum heat activation results in germination and colony formation by about 50% of an initial spore population. However, they reported that in the presence of 0.5N hydrochloric acid at 25°C the colony count increased to equal the direct microscopic count, accompanied by progressive release of DPA. Apparently activation by hydrochloric acid did not introduce lethality in these experiments, as is common with sub-lethal heat activation. Lewis et al. (1965) reported that treatment of B. stearothermophilus spores at pH 1.5 for 80 min at 25°C increased the colony count, but did not release DPA.

Sub-lethal heat treatments to activate B. stearothermophilus spores vary widely in time-temperature combinations, including 30 min. at 100°C (Kimsey et al., 1981; Davies et al., 1977), 10 min at 115°C (David and Merson, 1990), and 4 hours at 100°C (Srimani and Loncin, 1980; Srimani et al, 1980). In all cases, the aim of the workers was to obtain maximum spore counts.

2.7 Non-Logarithmic Behavior of Survivor Curves

Survivor curves have often deviated from logarithmic behavior, especially with highly dormant and heat resistant spores such as B. stearothermophilus (Shull et al., 1963; Davis et al., 1977; Kalinina and Motina, 1984; Abraham et al. 1990). Although initially these deviations were attributed to heat lags by Ball and Olson (1957), later workers concluded they were characteristic of bacterial spores (Humphrey and Nickerson, 1961). Fig. 2.7 illustrates the common deviations (curves II, III, and IV) from linearity (Curve I). The most commonly accepted



2.7 Types of survivor curves

explanation for the biphasic curve (Curve II in Fig. 2.7) is existence of a spore sub-population with heat resistance appreciably higher than the rest of the population (Davies et al., 1977; Cerf, 1977). A biphasic curve can be explained by two parallel reactions of logarithmic order having different rate constants (K_1 and K_2):

$$N = N_1+N_2 = N_{01}e^{-K_1t}+N_{02}e^{-K_2t} \qquad (2.20)$$

Curve III expresses a lag in the destruction of microorganisms when compared with first-order kinetics. It can be explained by the multiple hit theory, which proposes that several heat sensitive sites within a spore must be inactivated before the organism is inactivated. The average number of sites can be estimated by extrapolating the logarithmic portion of a survival curve back to the ordinate. A multi-hit model can be expressed mathematically by the following equation:

$$N = N_0[1-(1-e^{-Kt})^n] \qquad (2.21)$$

where n is the number of sites (Alper, 1960).

Humphrey and Nickerson (1961) expanded the multiple hit theory with the proposal that lag was due to several heat induced, heat labile reproduction initiators in the spore. During exposure to heat, an initiator is converted from an inactive, less heat labile state to a sensitive state. Such conversion is analogous to transition of a spore from dormant to activated. Inactivation involves denaturation of all initiators. Using B. stearothermophilus data, they estimated between 4 to 10 initiators for these spores. This model would also be adequate to describe the behavior of a nonhomogeneous suspension in which aggregates of two or more spores co-exist with isolated spores. In Eq. (2.20) n may express the mean number of spores in an aggregate.

In type IV curves, the number of countable spores increases at the beginning of a heat treatment. Because the logarithmic portion of the survivor curve did not extrapolate to the spore population observed by

direct count, Halvorson (quoted in Shull et al. (1963)) reasoned that activation alone was not completely responsible for this increase in population. He suggested that the initial shape of the curve could be attributed partly to heat activation of dormant spores and partly to a "multiple-hit" inactivation process. However, Shull et. al (1963) argued that this hypothesis would hold true only if nonactivated spores and activated spores were of equal heat resistance. Yet, if heat activation preceded thermal death or if nonactivated spores were of greater resistance than activated spores, a time delay would result before all remaining viable spores were activated and became equally susceptible to heat. In that case the logarithmic curve would extrapolate to a point in excess of the direct count, as Halvorson had observed.

Shull et al. (1963) assumed that a significant fraction of an original population (direct microscopic count) was in the dormant state, and developed a mathematical model to account for the characteristic shoulder of curve IV. They postulated that the transition from dormancy to the activated state and, finally, to the dead state was sequential and could be represented by two first order differential equations. They expressed the response of the activated spore population mathematically by the following equation:

$$N_2(t) = N_{20}e^{-K_d t} + N_{10}\frac{K_a}{K_a - K_d}(e^{-K_d t} - e^{-K_a t}) \qquad (2.22)$$

where K_a and K_d are rate constants for the activation and inactivation reactions, and N_{10} and N_{20} are initial numbers of dormant and active spores respectively, in the suspension. Upon applying this equation to B. stearothermophilus data, they found the model emulated the shape of the data curve, but had critical differences, especially during early moments of a heat treatment, which they attributed to experimental error. This hypothesis of consecutive reactions was studied later by Lewis et al. (1965). Using data for B. coagulans, they concluded that

no reason could be found to justify sequential coupling of the two reactions. However, Abraham et al. (1990) recently dealt with the Shull model in terms of dormancy rate (N_{10}/N_{20}) and initial velocities $d(N_2/N_{20})/dt$, to explain the behavior of their experimental curves with B. stearothermophilus and other shapes (linear and biphasic) of survivor curves found in the literature. By application of the Shull model to experimental data and considering dormancy rate to be temperature independent, Abraham et al., (1990), showed that the limiting reaction was the activation reaction. Kalinina and Motina (1984) also considered a sequential reaction model for B. stearothermophilus spores. They assumed that upon heating, activated spores in the sample perished immediately. This was followed by activation of dormant spores and subsequent inactivation of these spores. Recently, Rodriguez et al. (1988) predicted the dynamics of bacterial spore populations under lethal heat treatment by developing a composite model. The Rodriguez model was the basis for the present work and is presented in detail below.

2.8 Conceptual Model Proposed by Rodriguez et al. (1988)

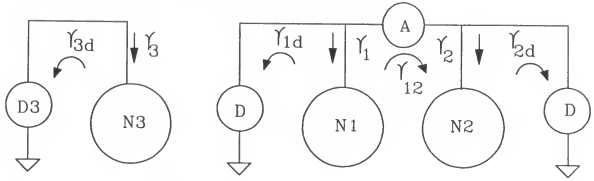
A conceptual model of a suspension of bacterial spores at lethal temperature was proposed by Rodriguez et al. (1988). This model included simultaneous and independent activation and inactivation transformations acting on dormant, viable spores and an inactivation transformation acting on spores in a less-heat resistant population (Fig 2.8). Triangles represented sinks for inactivated spores. Mature dormant spores comprised the population store N_1 and activated spores were represented by store N_2. Less-heat resistant populations were represented by store N_3. Activation, depicted by transformation A, transferred dormant, viable, mature spores in store N_1 to the activated population in store N_2. Transformations, D, depicted inactivation of populations in N_1 and N_2, and transformation D_3, depicted inactivation of

Transformations

D: Inactivation

A: Activation

D3: Inactivation of less-heat resistant spores



Stores

N1: Viable Dormant Spores

N2: Activated Spores

N3: Less heat resistant spores

2.8 System diagram of bacterial spore population (Rodriguez et al., 1988)

less-heat resistant fraction N_3. Stores and transformations were described and a mathematical model was formulated yielding rates of change of populations in the stores. It is important to mention that this model made no distinction between inactivation of dormant and activated spores and assumed similar transformations, D, for these spores. The components of the model were described as follows:

Stores:

$$N_1: \quad \frac{dN_1}{dt} = \Upsilon_1 \tag{2.23a}$$

$$N_2: \quad \frac{dN_2}{dt} = \Upsilon_2 \tag{2.23b}$$

$$N_3: \quad \frac{dN_3}{dt} = \Upsilon_3 \tag{2.23c}$$

Transformations:

Activation:

$$A: \quad \Upsilon_{12} = K_a N_1 \tag{2.24}$$

Inactivation:

$$D: \quad \Upsilon_{jd} = K_d N_j; \quad (j=1,2) \tag{2.25a}$$

$$D_3: \quad \Upsilon_{3d} = K_{d3} N_3 \tag{2.25b}$$

Coefficients K_a, K_d, and K_{d3} are rate constants. Since a node cannot store, the summation of flows at every node is zero.

Summation of flows at node a:

$$-\Upsilon_1-\Upsilon_{1d}-\Upsilon_{12} = 0 \quad \rightarrow \quad \Upsilon_1 = -(\Upsilon_{1d}+\Upsilon_{12}) \tag{2.26a}$$

Summation of flows at node b:

$$\Upsilon_{12}-\Upsilon_2-\Upsilon_{24} = 0 \quad \rightarrow \quad \Upsilon_2 = \Upsilon_{12}-\Upsilon_{2d} \tag{2.26b}$$

Summation of flows at node c:

$$\gamma_{3d} - \gamma_3 = 0 \quad \rightarrow \quad \gamma_3 = -\gamma_{3d} \tag{2.26c}$$

Rearranging Eqs. (2.25 through 2.30) and substituting in Eqs. (2.22 through 2.24) yields the mathematical model

$$\frac{dN_1}{dt} = -(K_d + K_a) N_1 \tag{2.27a}$$

$$\frac{dN_2}{dt} = K_a N_1 - K_d N_2 \tag{2.27b}$$

$$\frac{dN_3}{dt} = -K_{d3} N_3 \tag{2.27c}$$

The analytical solution of the mathematical model at constant temperature is

$$N_1(t) = N_{10} e^{-(K_d + K_a)t} \tag{2.28a}$$

$$N_2(t) = (N_{10} + N_{20}) e^{-K_d t} - N_{10} e^{-(K_d + K_a)t} \tag{2.28b}$$

$$N_3(t) = N_{30} e^{-K_{d3} t} \tag{2.28c}$$

The initial condition of the j^{th} store is denoted by $N_{j0} = N_j(0)$. An analogous model was developed for injured populations. Parameters for the model and initial validation of it were obtained by isothermal experiments using B. subtilis A spores. The authors concluded that this model adequately described the dynamics of survivor spores treated at constant lethal temperature, and that the Arrhenius equation described the effect of temperature on the rate constants over the range tested (87-99°C).

2.9 Methods for Determining Thermal Resistance of Bacterial Spores

The thermal resistance of bacterial spores at lethal temperature is determined by indirect heating systems (capillary tubes, thermal death time tubes or cans) and direct heating systems (mixing methods, product into steam, steam into product and electrical heating). Relative merits of some of these techniques were reviewed by Stumbo (1973).

The indirect method consists of measuring the number of viable organisms in a suspension sealed in an ampoule or a capillary tube before and after heating at one constant oilbath or steam temperature. The sample is cooled immediately after heating. This technique is convenient, easy, and does not require very sophisticated equipment; however, one significant problem is the time lag of heat transfer encountered when the sample is heated from ambient temperature to the reaction temperature. This transient thermal regime could contribute significant thermal inactivation, especially at higher reaction temperature. Perkin et.al.(1977) studied the effect of heating period on the death of B. stearothermophilus spores heated in capillary tubes. They developed equations for calculating an equivalent time assuming instantaneous heating of the sample to reaction temperature. Their results showed that heating period has negligible effect for reaction temperature $\leq$ 135°C. Above that temperature, heating period became increasingly significant and analysis using equivalent time equations provided more reliable data. However, for less heat resistant spores like C. sporogenes where decimal reduction times are of the order 2-4 sec at 132-135°C (Pflug and Esselen, 1953), the effect of thermal lag around 135°C could be significant. Using a system of weights and pulleys, Stern and Proctor (1954) designed an apparatus to transfer capillary tubes automatically, thereby allowing for reproducible thermal lag.

Direct heating methods involve mixing small quantities of spores with larger quantities of hot liquids. Wang et. al. (1964) used a tubular flow reactor to study inactivation kinetics of B. stearothermophilus at high temperature. This method required large volumes of spore suspension and determination of the distribution of spore residence times through the reactor caused by the velocity distribution of flow inside the reactor.

A thermoresistometer (Stumbo, 1965) electro-mechanically transports spore samples into a steam chamber, maintained at constant temperature, and drops the heat treated samples into tubes of dilutant. This equipment uses small quantities of sample, and does not require determination of the distribution of spore residence times. However, the design of this equipment is complicated and expensive, and it is no longer manufactured commercially. Srimani et al. (1980) used a laboratory scale, tubular flow heat reactor to obtain D values of spores of B. stearothermophilus and B. subtilis at temperatures up to 145°C. David et al. (1990) designed a computer controlled reactor for studying high temperature reactions, including death of bacterial spores at temperatures up to 155°C and short durations on the order of seconds. Rapid heating of the spores was achieved by thinly spreading 10μl samples on hydrophilic filter paper disks exposed to steam. A piston carried the disks of spore suspension into a high temperature steam chamber, exposing the thin sample to steam on both sides for the desired holding time, which could be as short as 0.1 sec. The sample was withdrawn automatically and dropped into a chilled dilution tube for subsequent plating to enumerate survivors. Brown et al. (1988) designed a computer controlled thermoresistometer capable of operating between 100 and 150°C±0.1°C with exposure time as short as 0.1 sec. Five samples (0.01 ml) of spore suspension could be exposed to steam at each time/temperature combination using a computer-controlled piston-driven exposure system. Control of temperature in the apparatus was achieved

using platinum resistance probes linked to the computer, which then actuates a variable steam control valve.

CHAPTER 3

METHODOLOGY

This chapter describes the development of a new comprehensive mathematical model, referred to as the Sapru model, for predicting the response of bacterial spore populations subjected to lethal temperature. It also explains the experimental methodology for generating isothermal survivor curves from which rate constants of the model and the effect of temperature on them were obtained. Procedures for calculating the values of rate constants in this model from survivor curves are explained. Finally, this chapter describes experiments for validating the model with non-isothermal temperature histories in normal and UHT ranges.

3.1 The Sapru Model.

Rodriguez et al. (1988) proposed a model describing population dynamics of bacterial spores for biological validation of thermal sterilization processes. That model, discussed in detail in the preceding chapter, assumed simultaneous and independent activation and inactivation transformations affecting bacterial spores subjected to lethal temperature. The development of it assumed that inactivations of dormant and activated spores are identical transformations. However, activation induces many changes in spores, increasing germination rate, activating enzymes which are dormant in resting spores, and changing the requirement for induction of germination (Keynan and Halvorson, 1965). The authors hypothesized that activation reversibly changes the tertiary structure of one or more proteins controlling the dormant state of a spore. It has also been shown that activated spores of

B. stearothermophilus can be separated by buoyant density centrifugation into a separate band less dense than the dormant spore band (Beaman et al., 1988). This implies that activated spores differ significantly from viable, dormant spores, and it is prudent to hypothesize that inactivation of viable, dormant spores differs from that of activated spores. That hypothesis was the basis for the Sapru model investigated here.

The system diagram for the Sapru model is presented in Fig. 3.1. Dormant, viable spores potentially capable of producing colonies in a growth medium after activation comprise the population in store N_1. Activated spores capable of forming colonies in a growth medium comprise the population in store N_2. Activation, depicted by transformation A, transfers dormant, viable spores from N_1 to N_2. Inactivated spores, incapable of germination and growth, comprise the populations in stores N_0 and N_3. Inactivation transformation D_1 transfers viable, dormant spores from N_1 to N_0. Inactivation transformation D_2 transfers activated spores from N_2 to N_3. It is assumed that transformations A, D_1, and D_2 are independent, concomitant, and first-order reactions.

3.1.1 Mathematical model

The equations describing rates of change of populations in stores N_0, N_1, N_2 and N_3 along with their initial conditions are:

$$\frac{dN_0}{dt} = K_{d1}N_1; \qquad N_0(0) = N_{00} \tag{3.1a}$$

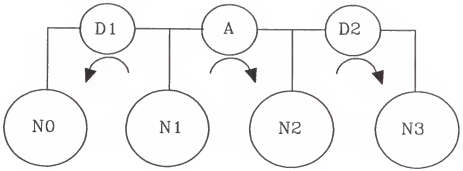
$$\frac{dN_1}{dt} = -(K_{d1}+K_a)N_1; \qquad N_1(0) = N_{10} \tag{3.1b}$$

$$\frac{dN_2}{dt} = K_aN_1 - K_{d2}N_2; \qquad N_2(0) = N_{20} \tag{3.1c}$$

$$\frac{dN_3}{dt} = K_{d2}N_2; \qquad N_3(0) = N_{30} \tag{3.1d}$$

Transformations

D1: Inactivation of dormant spores
D2: Activation of dormant spores
D3: Inactivation of activated spores



Stores

N0: Spores inactivated without activation
N1: Viable Dormant Spores
N2: Activated Spores
N3: Spores inactivated after activation

3.1 The proposed system diagram of bacterial spore population

Coefficients K_s, K_{d1} and K_{d2} are the specific rate constants of the transformations.

3.1.2 Response equations

Analytical solution of the above equations at constant temperature with the indicated initial conditions yields the following isothermal response equations (Appendix I):

$$N_0(t) = N_{00} + N_{10}\frac{K_{d1}}{K_{d1}+K_s}(1-e^{-(K_{d1}+K_s)t}) \tag{3.2a}$$

$$N_1(t) = N_{10}e^{-(K_{d1}+K_s)t} \tag{3.2b}$$

$$N_2(t) = (N_{20}+N_{10}\frac{K_s}{K_{d1}+K_s-K_{d2}})e^{-K_{d2}t} - N_{10}\frac{K_s}{K_{d1}+K_s-K_{d2}}e^{-(K_{d1}+K_s)t} \tag{3.2c}$$

$$N_3(t) = N_{30} + (N_{20} + N_{10}\frac{K_s}{K_{d1}+K_s-K_{d2}})(1-e^{-K_{d2}t}) - N_{10}\frac{K_{d2}K_s}{(K_{d1}+K_s-K_{d2})(K_{d1}+K_s)}(1-e^{-(K_{d1}+K_s)t}) \tag{3.2d}$$

Members of dormant, viable spore population N_1 are transferred by activation to N_2, adding to its initial, activated population, and cause the increase in survivors often observed early in a heat treatment. As population N_1 depletes due to simultaneous activation and inactivation of its members, inactivation of activated spores in N_2 begins to dominate, causing the observed transition zone (shoulder) and subsequent decline of the survivor population. The resulting exponential decay of N_2 corresponds to the observed tail of a survivor curve and is what the conventional, single-term model describes. Wang et al. (1964), observed that exponential decay only approximated the survivor dynamics in this high-kill region for populations of B stearothermophilus spores subjected to high lethal temperature in a tubular flow reactor.

Only activated spores N_2 will produce colonies on growth media, and their response is described by Eq.(3.2c).

When the initial number of dormant, viable spores $N_{10} = 0$, the response equation (3.2c) for activated spores reduces to the conventional, single-term model:

$$N_2(t) = N_{20}e^{-K_{d2}t} \tag{3.3}$$

This situation arises when a spore suspension has been uniformly activated by heat shock applied prior to lethal heat treatment.

If inactivation applies only to activated spores and can only follow activation of dormant spores without any inactivation of dormant spores, then $K_{d1} = 0$, and the response equation (3.2c) reduces to that derived by Shull et al. (1963):

$$N_2(t) = (N_{20} + N_{10}\frac{K_a}{K_a - K_{d2}})e^{-K_{d2}t} - N_{10}\frac{K_a}{K_a - K_{d2}}e^{-k_a t} \tag{3.4}$$

If dormant as well as activated spores can inactivate and if the rates of inactivation are identical, i.e., $K_{d1} = K_{d2}$, then the response equation (3.2c) reduces to that given by Rodriguez et al. (1988):

$$N_2(t) = (N_{20}+N_{10})e^{-K_{d2}t} - N_{10}e^{-(K_{d2} + K_a)t} \tag{3.5}$$

Note how these equations differ from survivor response, N_2, given by Eq.(3.2c) for the Sapru model.

3.2 Isothermal Experiments for Determination of Rate Constants

The dormant spore population cannot be measured directly, since they will not form colonies on growth media; however, once these spores have been activated, they will produce colonies on a growth medium. That fact is the basis for measurement of the number of survivors of a heat treatment. A survivor curve at constant temperature, is obtained by plotting the number of surviving, activated spores as a function of time. The response equation of any model is an attempt to describe or

predict the experimental survivor response curve of a real population for the stated thermal regime. The response of the activated spore population N_2 to lethal temperature is described by Eq.(3.2c) for the Sapru model, by Eq.(3.3) for the conventional, single-term model, by Eq.(3.4) for the Shull model, and by Eq.(3.5) for the Rodriguez model. In each case, rate constants of a model were determined by fitting the response equation of that model to the survivor data at that temperature. Analysis of rate constants from survivor data at various temperatures provided relationships describing the effect of temperature on them.

Rate constants of the Sapru, Rodriguez and Shull models were obtained from analysis of isothermal survivor curves at 105, 110, 115, and 120°C. At each temperature, the experiment was repeated three times to provide independent sets of survivor data. Capillary tubes containing spore suspension were heated at constant temperature for progressively longer time, withdrawn, and cooled. Spores were recovered from the capillaries, plated out on growth media, incubated to form colonies, and colonies were counted to estimate the survivor population at the time of withdrawal.

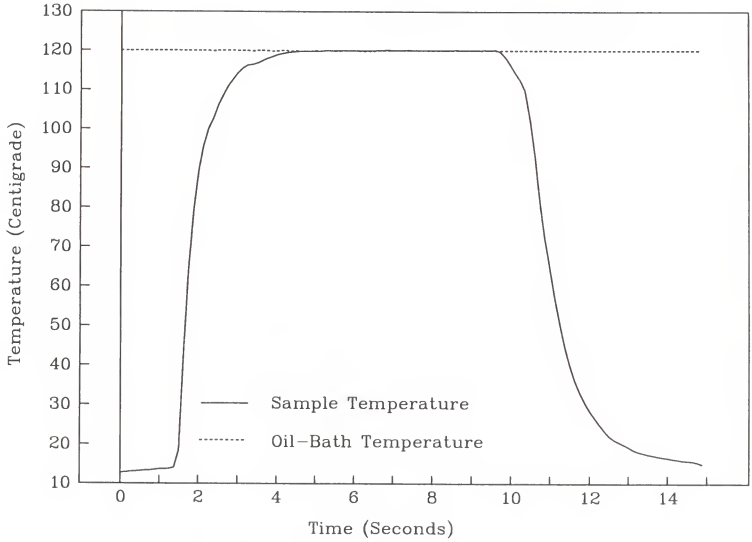
Dormant B. stearothermophilus spores (ATCC 7953: NCA 1518) more than four years old, were obtained from Dr. J.A Lindsay at the University of Florida, Department of Food Science and Human Nutrition. The spores were washed and suspended in 0.1M phosphate buffered saline (PBS), pH=7.3 (Appendix IV-A). When observed under a microscope, the spores were fully refractile and free from vegetative cells, phase dark spores, and clumps. Also these spores were never submitted to heat shock or any other kind of stress and, hence, it was assumed that all spores were viable. Direct microscopic count (DMC) of the suspension was done twice at different times, using a Spencer bright-line hemacytometer (American Optical, Buffalo, NY). Initial number of active spores N_{20} was determined by the counting colony forming units of

untreated spore suspensions. Initial number of dormant spores N_{10} = DMC-N_{20}, since it was assumed that all spores were viable. The same spore suspension without further dilution was used throughout this investigation.

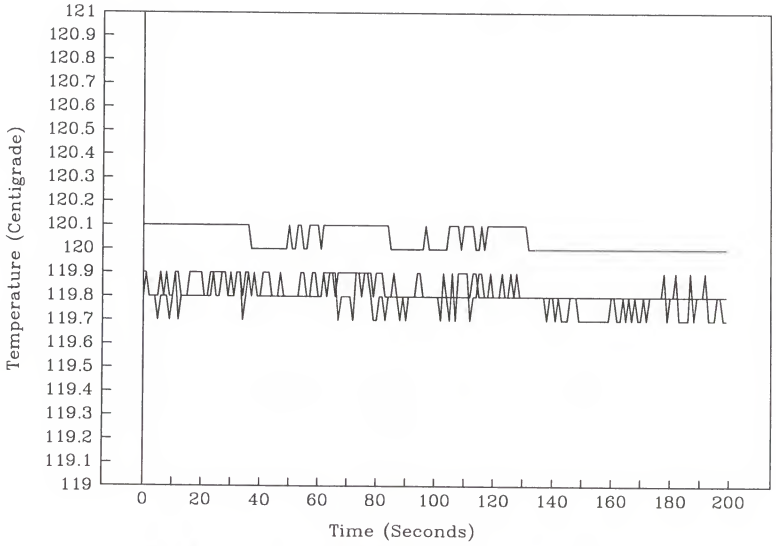
Samples (50μl) of spore suspension were micro-pipetted into capillary tubes fabricated from narrow ends of Pasteur pipettes and sealed by flame. It was necessary to inject the spore suspension into the capillary by a micropipette so as to prevent the sample from sticking to the tip. Best results were obtained when a micropipette tip end was flush with the capillary end. Spore containing capillary tubes were attached to wire holders and subjected to lethal temperature by manually placing and recovering them from a well stirred propylene glycol oil-bath, maintained at constant temperature (±0.1°C) (Lauda heating circulator model MT-6, Brinkman Instruments Co., Atlanta, GA).

Fig. 3.2 indicates the temperature experienced by a spore suspension manually immersed in and removed from an oil-bath at 120°C as measured by a thermocouple sealed in an identical capillary tube immersed beside the spore capillary. This was the maximum temperature for isothermal experiments, and since the minimum sampling time at this temperature was 15 seconds, Fig. 3.2 indicated it was safe to assume ideal square, temperature profiles in this investigation. It followed that negligible activation and inactivation of spores occurred during the time required for the sample of spore suspension to reach the experiment temperature. Fig. 3.3 depicts actual temperature at three different locations in the oil-bath set at 120°C and indicates spatial variation of temperature from the set point was within ±0.2°C, which was the maximum variation in temperature experienced by the sample. Temperatures were recorded on a CR10 datalogger (Cambell Scientific Inc., Logan, Utah).

Capillaries were manually withdrawn, cooled in ice-water, cleaned by immersing them in 70% ethyl alcohol for at least 10 minutes, and then



3.2 Sample temperature on immersion in the oilbath



3.3 Temperature at three different locations in the oilbath

washed with three changes of sterile distilled water. Samples were recovered by pulverizing the capillaries in glass tubes containing glass beads and dilutant (PBS). Pulverizing the capillaries rather than using the conventional wash out technique prevented loss of any sample. Abrasive action of glass beads does not cause reduction in survivor levels (Davies et al, 1977).

A sample (50μl) of the resulting suspension, after suitable decimal dilutions in 0.1M PBS, was pipetted in triplicate onto single dried plate of solidified nutrient agar medium (Becton Dickinson Microbiology Systems, Cockeysville, MD; Appendix IV-B). Micro-plating was used because it yielded reproducible counts that were higher than those obtained with spread plates that tend to lose spores due to attachment to the glass rod. This method also permitted more replicates per petri dish, thus reducing requirements of media, plates and time. Nutrient agar was used as the growth medium because in comparison to other media it gave the lowest initial counts of active spores and the highest counts after heat treatment. A low initial count of activated survivors implied the medium itself was not responsible for activating spores, while high counts after heat treatment of spore samples implied the medium was rich enough to promote recovery of injured spores. This was important because it is well known that thermal stress at high temperature can injure rather than kill spores.

Injured cells are fastidious for several nutritional factors as a consequence of partially damaged biosynthetic machinery, and resuscitation of injured cells requires a richer medium for recovery (Foegeding and Busta, 1981). A number of media are recommended by different investigators for identification of flat sour organisms (Labbe, 1979; Mallidis and Scholefield, 1986). However, the medium recommended by one group of workers yielded poor recovery when tested by other workers. Other media tested in this investigation included nutrient agar (Difco laboratories, Detroit, MI), brain heart infusion

(Difco), minimal media (Appendix IV-C), and combinations of casamino acids (Difco), nutrient agar, and glucose. For long exposure times, when very low colony counts were expected, the capillaries were emptied directly onto the solidified nutrient medium by clipping the ends using flamed pliers. These plates were wrapped in foil and incubated at 58°C for 12 hours. The resulting micro-colonies were then counted. Longer incubation time was not necessary since it did not increase the colony counts.

To provide accurate estimates of the rate constants, about 15 data points, well spaced over the duration of the survivor curve, were obtained at each temperature by subjecting spore suspension samples to heating times appropriately spaced in time. More data points were required to describe the rapidly varying shoulder than were required for describing the slowly varying straight line portion of the semi-log plot of a survivor curve. The duration of the straight line portion of a semi-log plot of survivor data (tail), required for estimation of the inactivation rate constant for activated spores by linear regression, was set to permit reduction of the maximum viable population by 6 to 8 log cycles, thereby avoiding any effect of activation on the decline of the population in this region.

3.3 Analysis of Survivor Curves for Determination of Rate Constants

3.3.1 Analysis of survivor curves using the Sapru model

Survivor curves were analyzed to obtain rate constants with nonlinear regression procedure (SAS, 1985). The nonlinear regression procedure was performed on the aggregate data set consisting of three replicate survivor curves at each temperature. The procedure used the modified Gauss-Newton iterative method to regress residuals onto the partial derivatives of the response with respect to the rate constants until the iterations converged. Initial estimates of rate constants required by the nonlinear regression procedure were obtained by the

method of successive residuals. The method of successive residuals is applicable when a response is a summation of exponentials with significantly different time constants. The time constant of an exponential determines its rate of decay. After 5 time constants of the faster exponentials, the slowest exponential in the sum dominates, and it can be determined by regression. Once determined, this exponential term can be removed from the experimental data to leave the other exponentials. This procedure is applied successively until the residual after removal of the last exponential is negligible.

Eq.(3.2c) can be written as:

$$N_2(t) = Ae^{-K_{d2}t} - Be^{-(K_{d1}+K_s)t} \tag{3.6a}$$

$$where \quad A=N_{20}+B \; ; \; B=N_{10}\frac{K_s}{K_{d1}+K_s-K_{d2}} \tag{3.6b}$$

Estimates of A, K_{d2}, B, and $(K_{d1}+K_s)$ in Eq.(3.6a) were obtained for each of the 4 constant temperatures using the method of successive residuals. The straight line portion of the semi-log plots of the mean survivor curve at each temperature, where the exponential term with K_{d2} dominates, was identified by inspection, and A and K_{d2} were obtained by linear regression of that portion of the semi-log plot of the mean survivor data at that temperature. Once the exponential term with K_{d2} was determined, the other exponential term in the response equation was found by subtraction, i.e.,

$$Be^{(-(K_{d1}+K_s)t)} = A\,e^{-K_{d2}t} - N_{20} \tag{3.7}$$

Initial estimates of K_s and K_{d1} were calculated from Eq.(3.6b) with $B = A-N_{20}$, and K_{d2}, and $(K_{d1}+K_s)$ known.

Pascal program "Residual" (Appendix III-A) was written to implement the method of successive residuals. Input to that program was a datafile containing mean experimental survivor curve for each

temperature. Initial estimates of rate constants obtained by the program "Residual" were then used in the nonlinear regression procedure to determine the final estimates of the rate constants K_{d1} and K_s. Final estimates of K_{d2} were obtained by linear regression of straight line portion of the semi-log plots of the aggregate survivor curve at each temperature.

Activation rate constant K_s and inactivation rate constant K_{d1} were obtained from the best fit of the response equation to the aggregate survivor curve at each temperature. Information provided for the nonlinear regression procedure (Appendix III-B) contained initial estimates of the rate constants, the response equation (Eq.(3.2c)) and its partial derivatives with respect to each rate constant. The estimation procedure was not confined by imposing bounds on the estimates. The nonlinear regression procedure consistently improved the values of rate constants, until the relative sum of squares error (SSE) was minimized, i.e.,

$$\frac{SSE_{i-1}-SSE_i}{(SSE+10^{-6})} < c \qquad (3.8)$$

where $c=10^{-4}$ is a small positive number. No data points were deleted.

3.3.2 Analysis of survivor curves using the Shull and Rodriguez Models

It was important to justify the existence of direct inactivation of dormant spores, as provided by transformation D_1 in the Sapru model, and to show that the rate constants of D_1 and D_2, the inactivation of activated spores, were distinct. This was done by comparing values of rate constants and responses of the Sapru model with those of the Shull and Rodriguez models. As mentioned earlier, the Shull model assumes that inactivation only follows activation of dormant spores. There is no inactivation of dormant spores. The Rodriguez model hypothesized inactivation of dormant spores but used the same inactivation rate constant for both dormant and activated spores. That rate constant

equaled the inactivation rate constant for activated spores found for the Sapru and Shull models.

Using response equations (Eqs.(3.4) and (3.5)) and the parameter estimation procedure described above, the aggregate survivor data at each of the four temperatures were analyzed to estimate activation and inactivation rate constants of the Shull and Rodriguez models. Predicted responses of these models were compared with responses (Eq.(3.2c)) of the Sapru model and of experiments.

3.3.3 Effect of temperature on the rate constants

To predict the responses of spore populations subjected to a dynamic temperature history, it was necessary to predict the values of the rate constants as functions of temperature in that history. Semi-log plots of activation and inactivation rate constants versus reciprocal absolute temperatures estimated from the Sapru model at the four isothermal temperatures indicated that the data could adequately be described by straight lines and the Arrhenius equation. Consequently, slopes and intercepts of those plots were used to calculate the activation energies and frequency constants in the Arrhenius Equation (Eq.(2.15)) for each transformation. Those equations were then used to determine rate constants at any temperature .

The effects of temperature on the activation rate constant for the Shull and Rodriguez models were examined, and it was found that the Arrhenius equation described the relationship adequately for both models. Recall that inactivation rate constants of activated spores are equal for all three models and also equal to the inactivation rate constant for dormant spores in the Rodriguez model.

3.4 Relative Sensitivity Analysis

Relative sensitivity of response N_2 to any parameter K, is defined by (Jones et al., 1987):

$$Relative\ Sensitivity = \frac{\delta N_2/N_2}{\delta K/K} \qquad (3.9)$$

The above equation was used as a normalized measure for comparing the sensitivity of response N_2 to rate constants of the Sapru model. Relative sensitivities of the model response were calculated for a 1% change in each rate constant at 120°C to establish the relative influence of that rate constant on the response of the model. Appendix III-C lists Pascal program "Sensitivity" that was used for this analysis.

3.5 Validation of Models for Non-Isothermal Temperature Histories

In two separate experiments, sealed capillary tubes containing 50μl of dormant B. stearothermophilus spores suspended in PBS were placed in the oil-bath and subjected to a varying temperature regime achieved by manual rotation of the temperature control on the oilbath. Tubes were removed at times spaced over the test interval and analyzed for survivor counts as described earlier. Samples were extracted more frequently at higher temperature than at lower temperature for better description of the faster moving survivor curve. The temperature histories of the oil bath was recorded by the CR10 datalogger four times per second.

Initial numbers of dormant spores, N_{10}, for the Sapru, Shull and Rodriguez models were obtained from the difference of direct microscopic count and average initial number of active spores, N_{20}, obtained from initial plate counts of untreated spores. For the conventional, single-term model, the initial spore population was set to equal the maximum activated spore count obtained by simulating the response of the Sapru model. This was justified because these counts were more than those that were obtained by heat activating spores at 100°C for 60 minutes, often used for heat activating spores of B stearothermophilus. The response of the single-term model was simulated from that point on.

In a third experiment, capillaries of spores heat activated conventionally (60 minutes at 100°C) were subjected to the dynamic temperature history of the second experiment. Experimental responses of these spores were compared to predicted responses of all the models. However, in this case the initial number of dormant spores, N_{10}, and activated spores, N_{20}, for the Sapru, Shull and Rodriguez models was obtained by simulating a uniform temperature treatment of 100°C for 60 minutes using the program "Survivors".

Pascal program "Survivors" (Appendix III-D) was written to predict the survivors of a dynamic temperature history recorded by the datalogger using the Sapru, Shull, Rodriguez and Conventional single-term models. The program implemented the response equation, N_s, of each model simulated and used the corresponding Arrhenius equations for estimating its rate constants from the temperature history. Starting with initial dormant and active populations appropriate to a model, the program calculated changes in and new spore populations for each time increment in the temperature history. Survivor populations predicted by each model over time were recorded in an output data file and plotted later along with the experimental data.

3.6 Extrapolation of rate constants to UHT range

It was important to test validity of the Arrhenius equation for temperature dependency of rate constants of Sapru model in the UHT range (125-150°C), especially since these equations were obtained from survivor data generated in the sub-UHT range (105-120°C). This was done by subjecting sample spore suspensions to ultra high temperature and comparing the final number of survivors predicted by the Sapru model with the final number of experimental survivors.

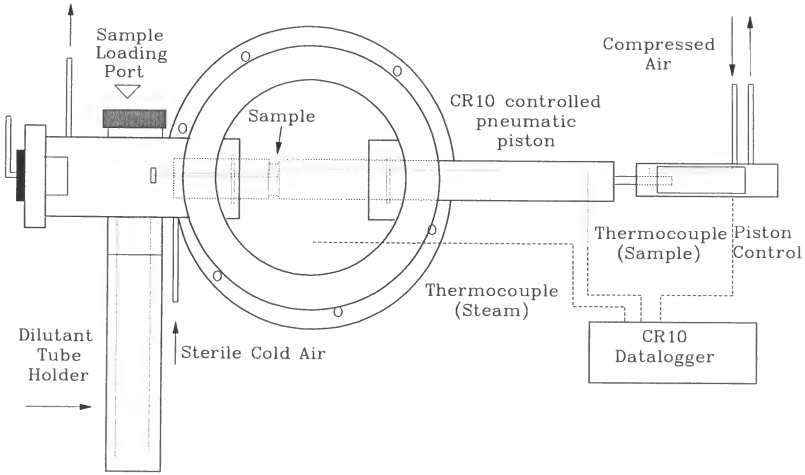
3.6.1 Capillary tube experiments

Sample spore suspensions in capillary tubes were subjected to ultra high temperature by immersing them in an oil filled beaker heated

with a bunsen burner to high temperature. The temperature history of the sample was measured by a thermocouple sealed with epoxy in an identical capillary tube immersed along with the sample capillary and recorded on the CR10 datalogger eight times per second. A total of twelve experiments were performed. For six of the twelve experiments, suspensions in two identical capillaries were immersed in the oil filled beaker, thereby providing duplicate readings, while each of the remaining six involved single capillary tube. After heat treatment, spore suspensions in the capillaries were analyzed as explained earlier to obtain the survivor counts at the end of each treatment. Theoretical survivors of the heat treatment were calculated as described in the previous section using the Sapru model in the Pascal program "Survivor". Since only one data point could be generated in each experiment, comparison of predicted and experimental survivor curves was not possible; the comparison was made of survivors at the end of the heat treatment for each experiment.

3.6.2 Steam reactor experiments

Considerable time was spent making a computer controlled reactor (David, 1985; David and Merson, 1990) obtained from University of California at Davis operational. The reactor, Fig. 3.4, had been used to determine kinetic parameters of B. stearothermophilus at high temperature. The reactor was bolted to a flange welded on the side of an existing retort (Dixie Canner Equipment Co., Athens, GA). Steam from a 60 psi line was regulated using a Taylor 440R series controller (Taylor instruments, Syracuse, NY) to maintain the desired reactor temperature. Sterile compressed air, bacteriologically filtered through hydrophobic filter paper (Durapore hydrophobic disc filter paper No. HVHP02500-25 mm disc filters, Millipore, Bedford, MA.) placed in a high pressure stainless steel holder (No. XX4502500, Millipore) was introduced into the antechamber via a cooling coil to maintain the sample at a low temperature prior to exposure to steam and to prevent



3.4 **Steam reactor setup**

possible flash evaporation of water from the sample on the reverse stroke. The air pressure was manually adjusted slightly less than the steam pressure in order to prevent cold, compressed air from leaking into the steam chamber.

The datalogger also controlled a relay that actuated the pneumatic piston of the reactor transporting a sample in and out of the steam chamber for a specified duration. Fig.3.4 depicts the equipment required for obtaining survivors of bacterial spores at elevated temperatures using this CR10 controlled reactor. Spore samples were directly subjected to lethal temperature by pipetting 10 or 25μl of vortexed spore suspension directly on hydrophilic filter paper (Durapore-HVLP 09050) sandwiched between hydrophobic filter papers (Durapore-HVHP 09050). The sample was held in place between two steel washers. Sampled temperature was recorded on a CR10 datalogger by inserting fabricated, type T copper/constantan thermocouples (Omega Engineering, CT) through the piston axis directly next to the sample. However, for the purposes of this investigation the loss of sample (10-20%) and the accumulation of condensate in the recovery tube prevented generation of reproducible survivor curves suitable for describing the shoulder of a survivor curve essential to our analyses. Attempts were made to account for the condensate by pre-weighing the recovery tubes, but the dispersion of the data was significant and prevented meaningful analysis of isothermal experiments. However, sample spore suspensions were subjected to non isothermal temperature histories. It is important to emphasize that this setup is adequate for the purpose of conventional experiments where high accuracy during the early moments of heat treatment are not essential.

CHAPTER 4

RESULTS AND DISCUSSIONS

This chapter presents rate constants of the Sapru, Shull and Rodriguez models obtained from nonlinear regression of survivor data at 105, 110, 115 and 120°C (Appendix II). Response curves for the three models were compared with experimental survivor data. Temperature dependency of rate constants is presented next, followed by validation experiments involving comparison of simulated responses using the Sapru, Shull, Rodriguez, and the conventional, single-term models with the experimental survivor data for non-isothermal temperature histories. Finally, numbers of survivors at the end of UHT experiments were compared with the numbers predicted by the Sapru model for those temperature regimes.

4.1 Estimates of Rate Constants

Three sets of survivor curves were obtained at 105, 110, 115, and 120°C (Appendix II). Initial number of dormant spores, $N_{10} = 3.3x10_9$, was obtained from the difference of direct microscopic count = $3.5x10^9$ (average of two separate measurements, $3.3x\ 10^9$ and $3.6x10_9$) and average initial number of active spores $N_{20} = 2.3x10^8$, obtained from initial plate counts of untreated spores.

4.1.1 Estimated rate constants for the Sapru model

Table 4.1 presents estimates of the predominant inactivation rate constant, K_{z2}, of activated spores, along with the correlation coefficient, R, for the Sapru model. The low correlation coefficient of K_{z2} at 105°C probably was due to dispersion in the survivor data. These

estimates of K_{d2} are equal to the inactivation rate constant for activated spores in the Shull, Rodriguez, and Conventional, single-term model, and to the inactivation rate constant for dormant spores in the Rodriguez model.

Table 4.1: Estimated rate constant K_{d2} and correlation coefficient R, for the Sapru, Shull, Rodriguez, and the conventional models.

Temperature	105°C	110°C	115°C	120°C
K_{d2}	0.017	0.072	0.297	1.38
R	0.93	0.97	0.97	0.99

Table 4.2 presents estimates of inactivation and activation rate constants K_{d1} and K_s of the Sapru model for dormant spores at each of the four temperatures. Also presented are the asymptotic standard error and lower and upper limits of the asymptotic 95% confidence interval.

Table 4.2: Estimated rate constants K_{d1} and K_s for the Sapru model.

Temperature	105°C	110°C	115°C	120°C
K_{d1}	0.018	0.022	0.068	0.432
Std. Error	0.004	0.008	0.053	0.118
Lower	0.011	0.005	-.039	0.194
Upper	0.025	0.039	0.174	0.669
K_s	0.016	0.063	0.307	1.167
Std. Error	0.002	0.007	0.034	0.113
Lower	0.013	0.050	0.238	0.938
Upper	0.020	0.077	0.376	1.396

The estimates of K_s have low standard error (standard deviation) and a narrow 95% confidence interval. However, such was not true of estimates of K_{d1}, probably due to dispersion in the survivor data.

Semi-log plots of experimental survivor data and regression curves for the Sapru model are presented for comparison in Fig. 4.1 through

Fig. 4.20. The response, N_2, predicted by the Sapru model, using rate constants presented in Table 4.1 and 4.2, describes the survivor data adequately. The agreement is evident in the semi-log plots of individual survivor curves as well as in the curves from aggregate data from 3 replications.

4.1.2 Estimated rate constants for the Shull and Rodriguez Models

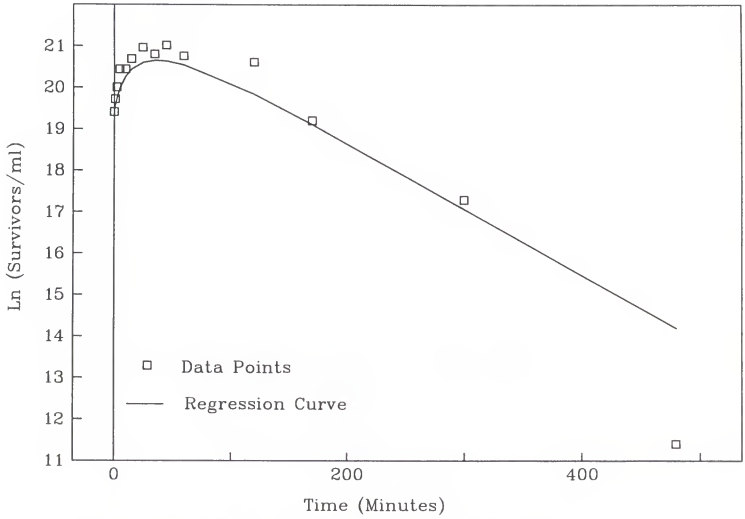
Table 4.3 presents the estimates of activation rate constant K_s for the Shull model and Rodriguez models along with the asymptotic standard error and lower and upper limits of the asymptotic 95% confidence interval.

Table 4.3: Estimated rate constant K_s for the Shull and Rodriguez models.

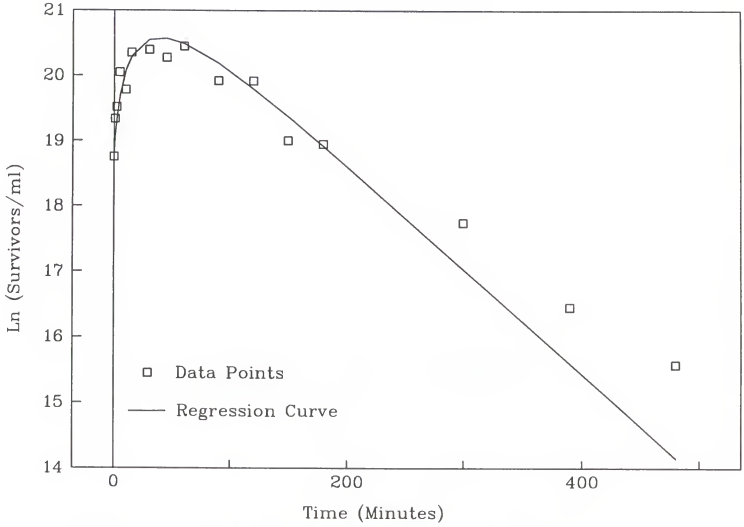
Shull Model:				
Temperature	105°C	110°C	115°C	120°C
K_s	0.0118	0.0596	0.3293	1.1377
Std. Error	0.0011	0.0055	0.0291	0.1034
Lower	0.0090	0.0480	0.2700	0.9287
Upper	0.0141	0.0708	0.3880	1.3467
Rodriguez Model:				
Temperature	105°C	110°C	115°C	120°C
K_s	0.016	0.088	0.405	1.611
Std. Error	0.001	0.009	0.038	0.173
Lower	0.013	0.070	0.328	1.260
Upper	0.017	0.105	0.481	1.960

These estimates of K_s for both the Shull and Rodriguez models have a low standard error and a narrow 95% confidence interval. The order of magnitude for this rate constant is comparable for all three models.

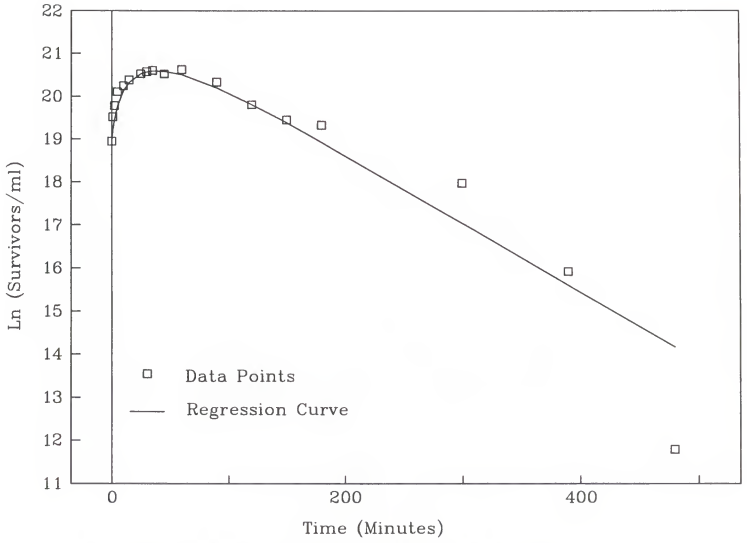
Semi-log plots of survivor data and regression curves for the Sapru, Shull and Rodriguez models are presented for comparison in Fig. 4.21 through Fig. 4.24. The number of survivors predicted by the Shull



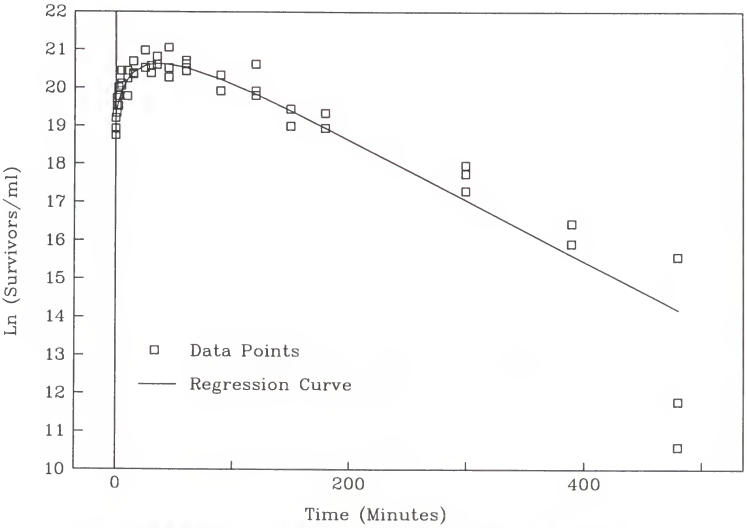
4.1 Survivor data and nonlinear regression curve at 105°C (i)



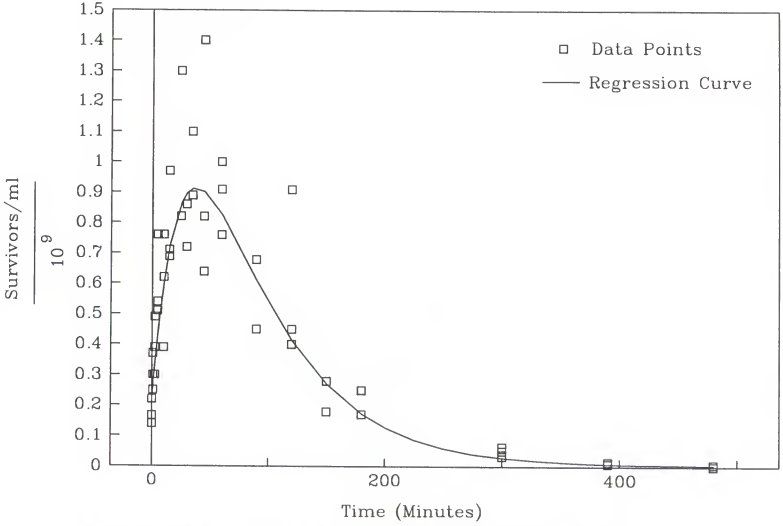
4.2 Survivor data and nonlinear regression curve at 105°C (ii)



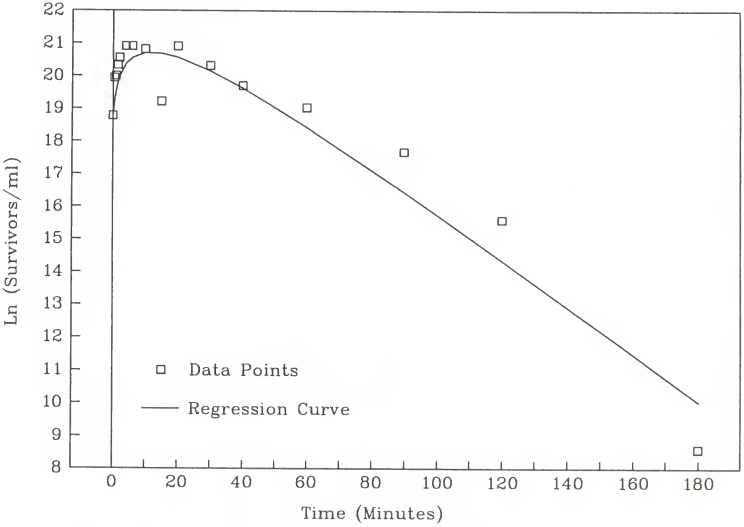
4.3 Survivor data and nonlinear regression curve at 105°C (iii)



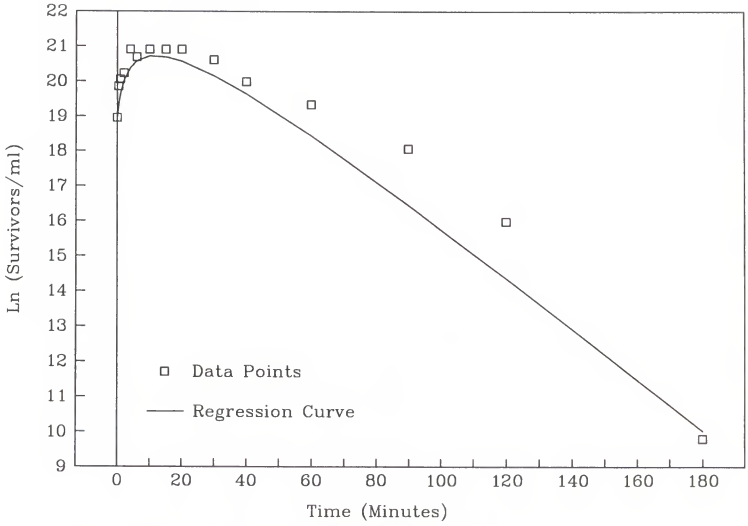
4.4 Aggregate survivor data and nonlinear regression curve at 105°C



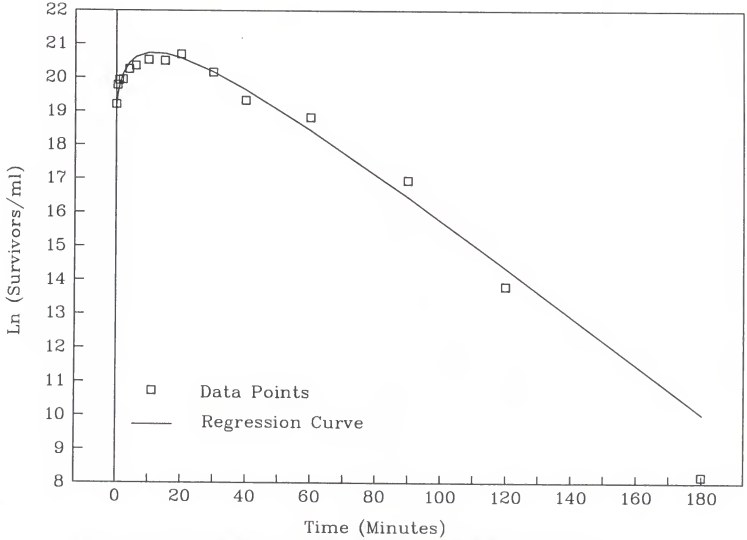
4.5 Absolute survivor data and nonlinear regression curve at 105°C



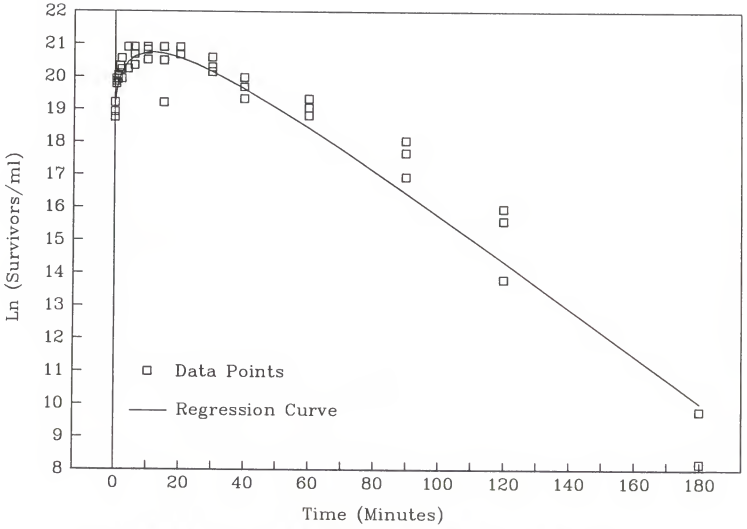
4.6 Survivor data and nonlinear regression curve at 110°C (i)



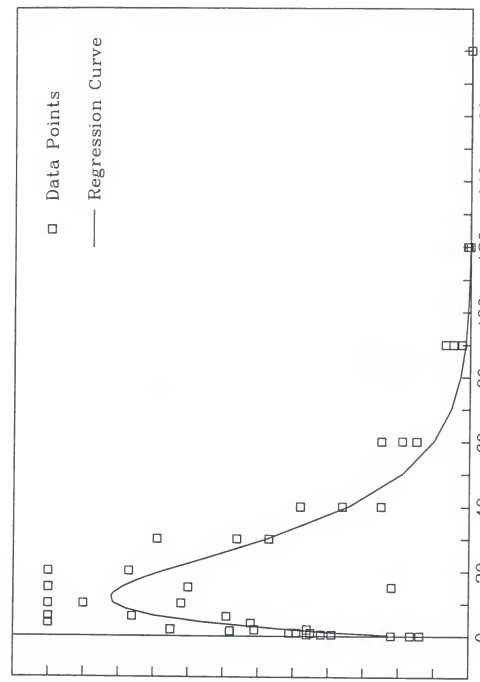
4.7 Survivor data and nonlinear regression curve at 110°C (ii)



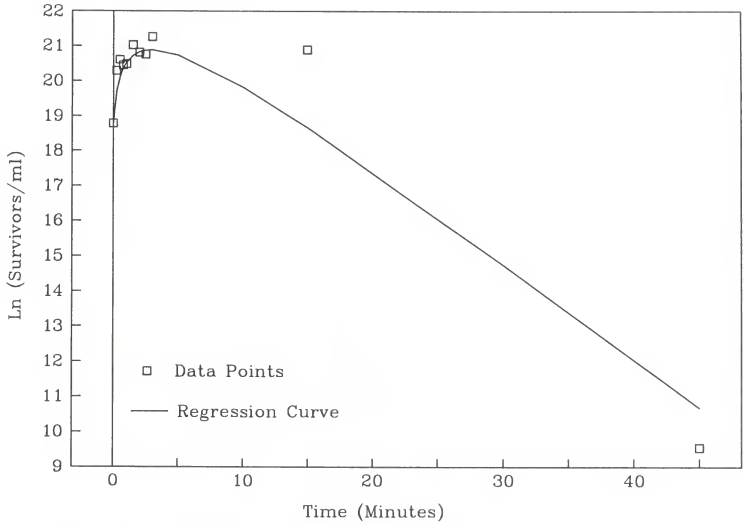
4.8 Survivor data and nonlinear regression curve at 110°C (iii)



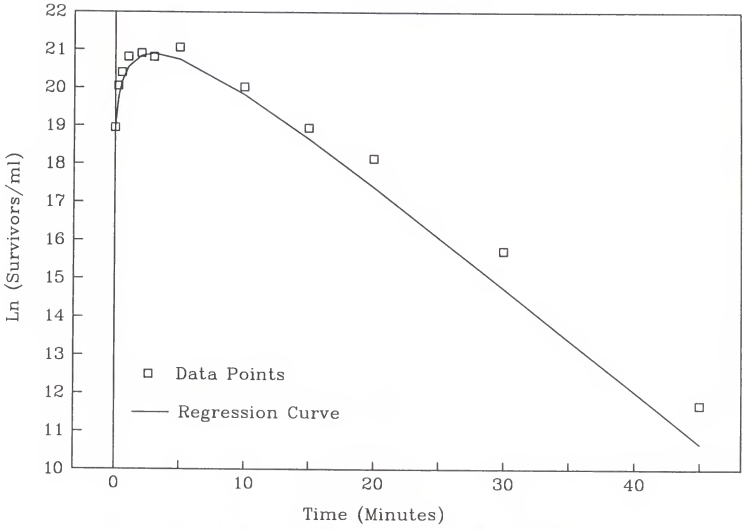
4.9 Aggregate survivor data and nonlinear regression curve at 110°C



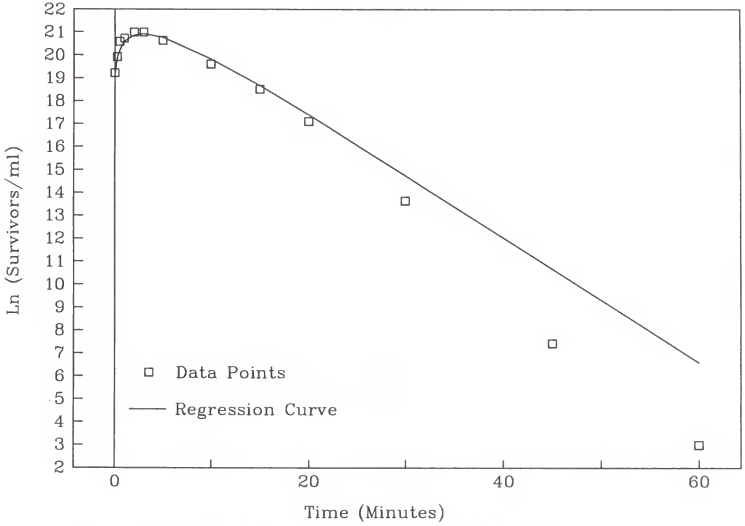
4.10 Absolute survivor data and nonlinear regression curve at 110°C



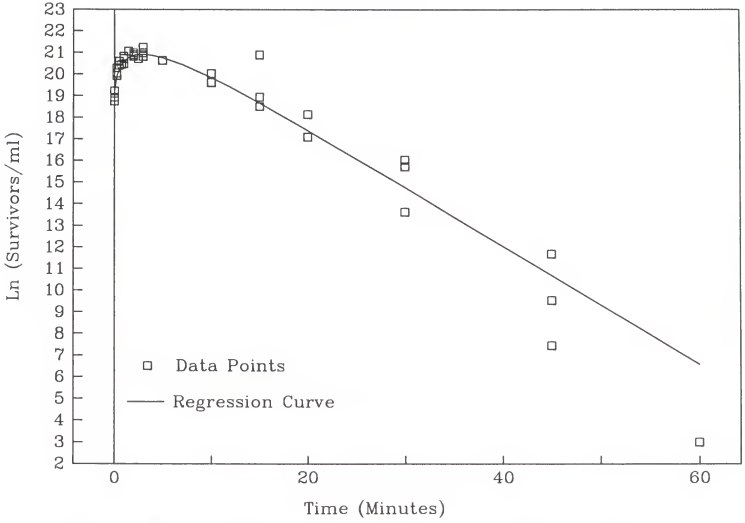
4.11 Survivor data and nonlinear regression curve at 115°C (i)



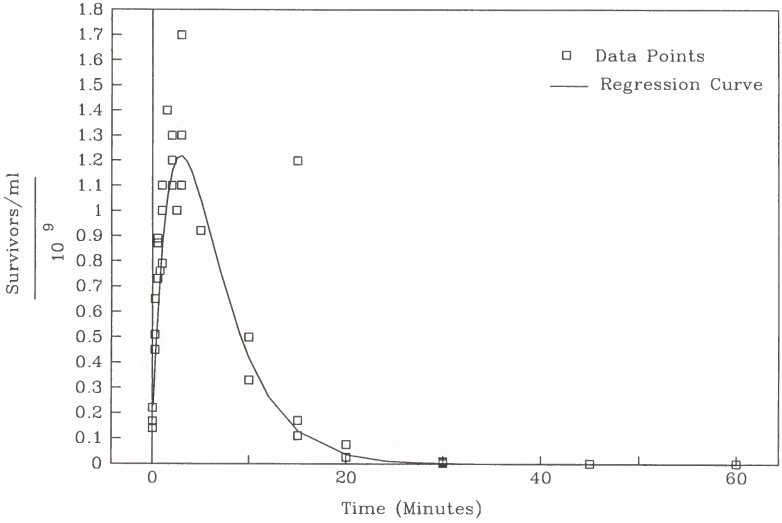
4.12 Survivor data and nonlinear regression curve at 115°C (ii)



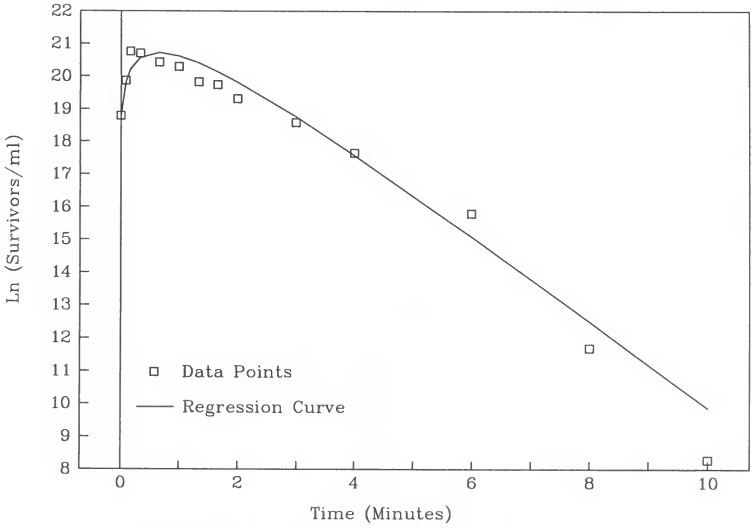
4.13 Survivor data and nonlinear regression curve at 115°C (iii)



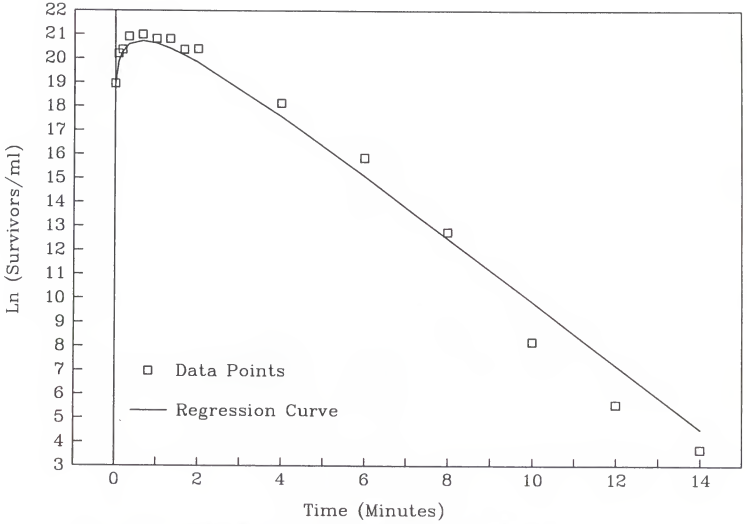
4.14 Aggregate survivor data and nonlinear regression curve at 115°C



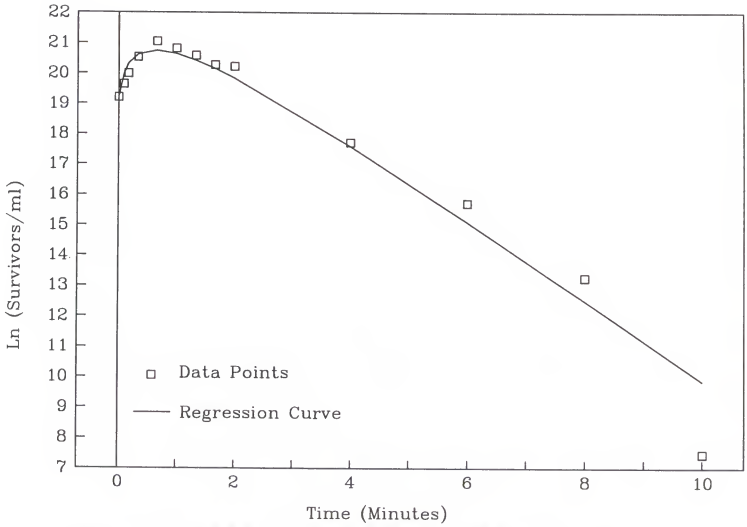
4.15 Absolute survivor data and nonlinear regression curve at 115°C



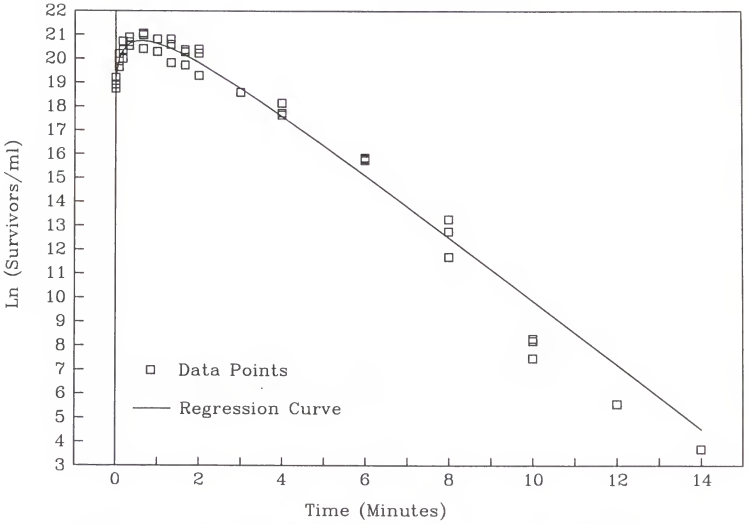
4.16 Survivor data and nonlinear regression curve at 120°C (i)



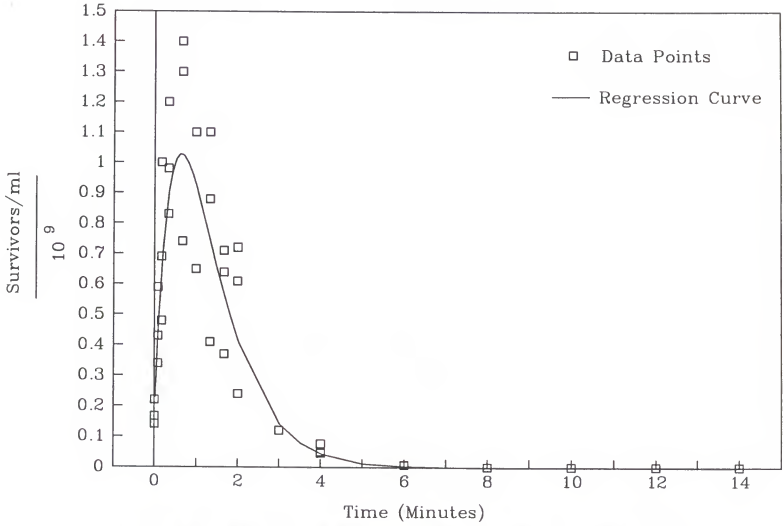
4.17 Survivor data and nonlinear regression curve at 120°C (ii)



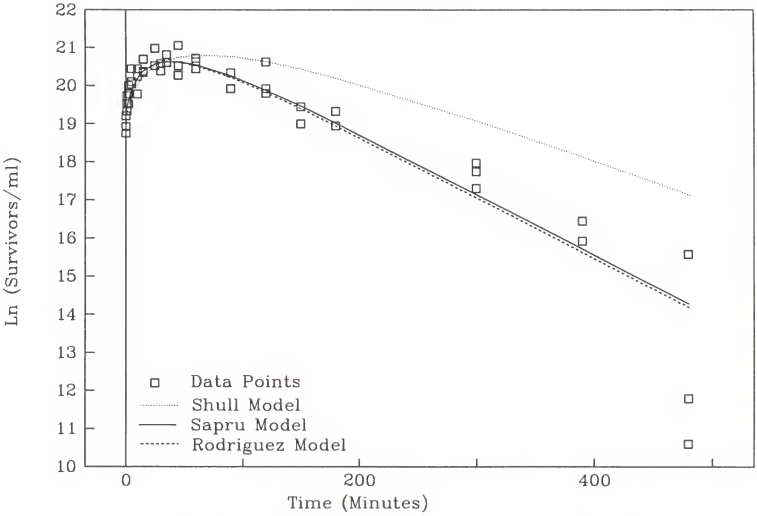
4.18 Survivor data and nonlinear regression curve at 120°C (iii)



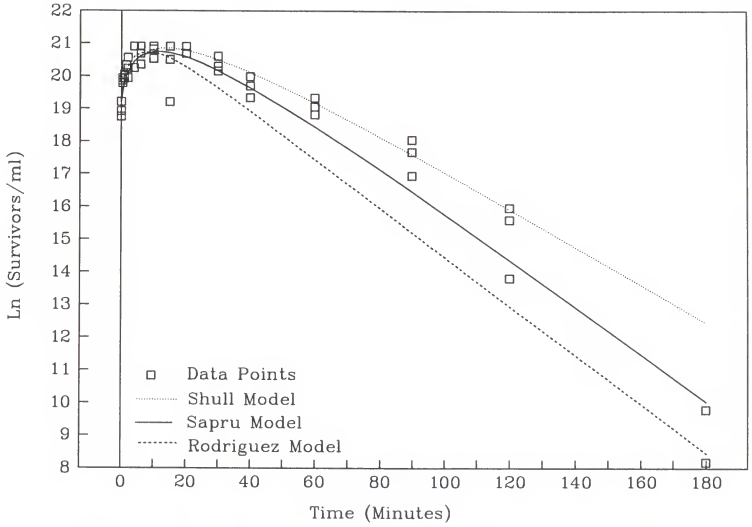
4.19 Aggregate survivor data and nonlinear regression curve at 120°C



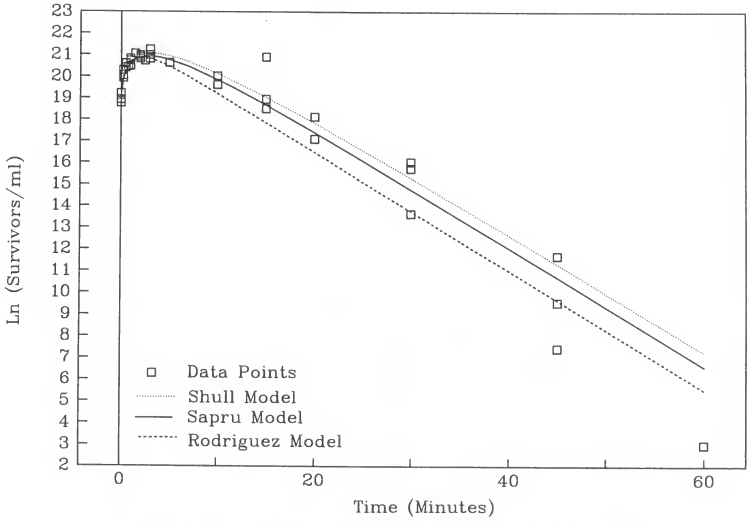
4.20 Absolute survivor data and nonlinear regression curve at 120°C



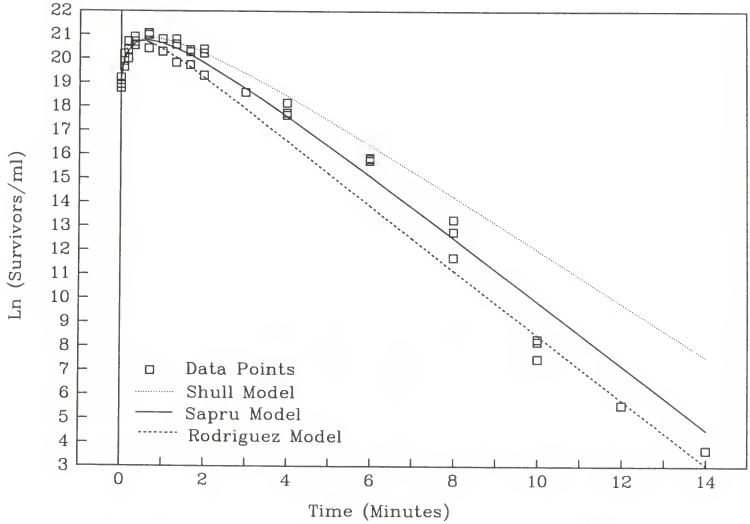
4.21 Comparison of Sapru model with Shull and Rodriguez models at 105°C



4.22 Comparison of Sapru model with Shull and Rodriguez models at 110°C



4.23 Comparison of Sapru model with Shull and Rodriguez models at 115°C



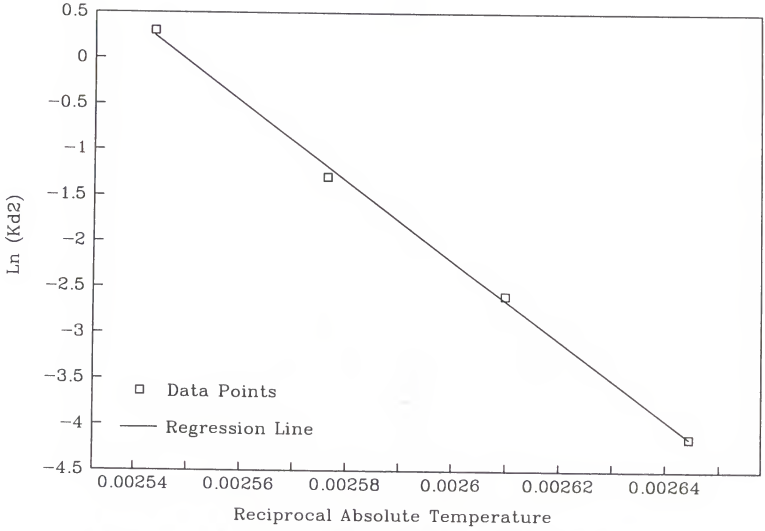
4.24 Comparison of Sapru model with Shull and Rodriguez models at 120°C

model is higher than the numbers predicted by the Sapru and Rodriguez models and are also greater than experimental numbers at all four temperatures. This deviation of the Shull model is more significant at 105°C than at higher temperatures. At that temperature, the values for rate constants K_{d1} and K_{d2} for the Sapru model are comparable, resulting in a significant inactivation of dormant spores; however, the Shull model does not include this transformation and, thereby, predicts a higher number of survivors.

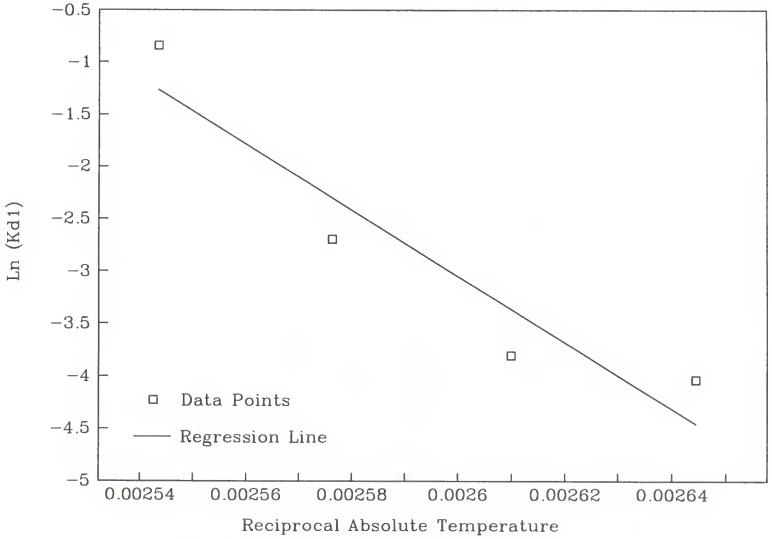
On the other hand the number of survivors predicted by the Rodriguez model are lower than the number predicted by the Sapru and Shull models and are also lower than the experimental survivors at all four temperatures. This deviation of the Rodriguez model is least significant at 105°C compared to higher temperatures. At this temperature, the values for rate constants K_{d1} and K_{d2} in the Sapru model are comparable and result in a response insignificantly different from that predicted by the Rodriguez model, which assumes these rate constants are same. However, at higher temperature, these differences in responses predicted by the Rodriguez and Sapru models are significant, because values of K_{d2} are higher than values of K_{d1} in the Sapru model. Inactivation of dormant spores in the Rodriguez model proceeds at a higher rate (K_{d2}) than in the Sapru model (K_{d1}), yielding fewer activated spores overall.

4.2 Effect of Temperature on Rate Constants.

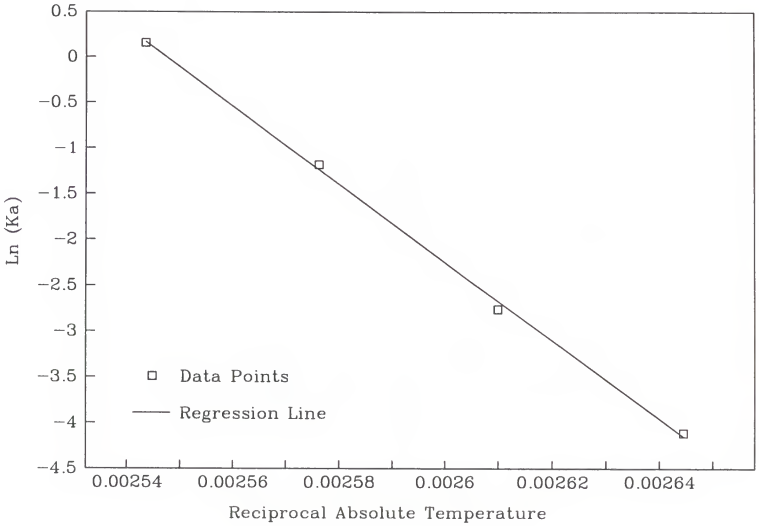
Figs. 4.25 through 4.28 are the Arrhenius plots (ln(K) vs. 1/T (°K)) of rate constants of the Sapru model, and Figs. 4.29 and 4.30 are the Arrhenius plots of the activation rate constants of the Shull and Rodriguez models, respectively, over the four isothermal temperatures. The plots were linear for all rate constants of the Sapru, Shull and Rodriguez models. Except for rate constant K_{d1} of the Sapru model, all plots had high correlation coefficients (R=0.99). The somewhat low correlation coefficient for K_{d1} (R=0.95) corresponds to the high standard



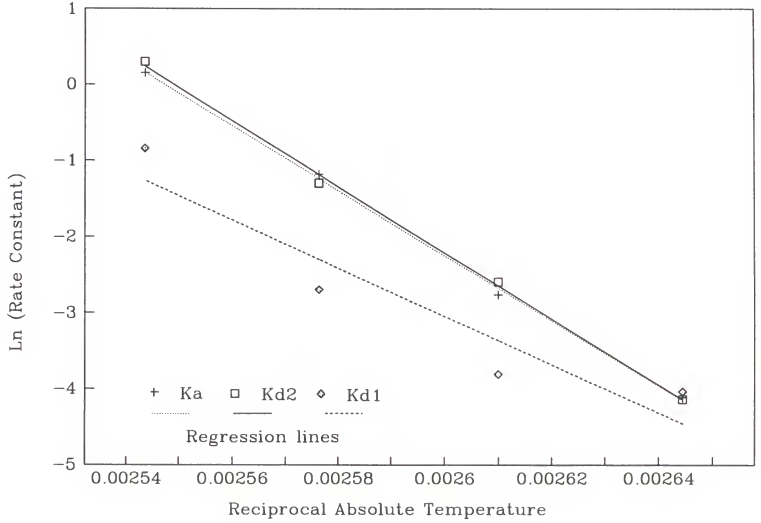
4.25 Arrhenius plot for inactivation rate constant K_{d2} (All Models)



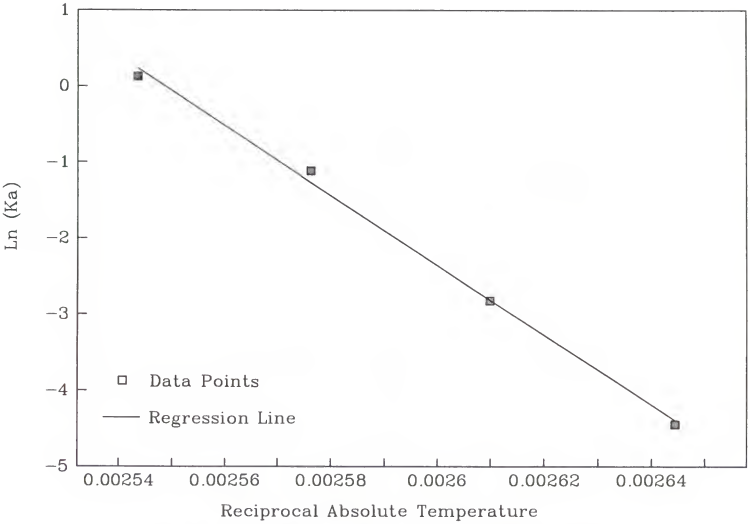
4.26 Arrhenius plot for inactivation rate constant K_{d1} (Sapru Model)



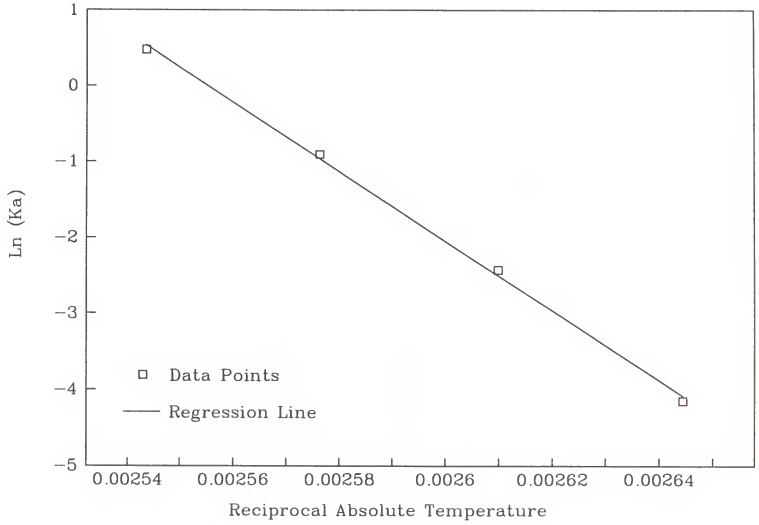
4.27 Arrhenius plot for activation rate constant K_a (Sapru Model)



4.28 Comparison of Arrhenius plots for rate constants (Sapru Model)



4.29 Arrhenius plot for activation rate constant K_a (Shull Model)



4.30 Arrhenius plot for activation rate constant K_a (Rodriguez model)

error and wide confidence interval in the estimates of this rate constant. These results indicated that the temperature dependence of all rate constants could be described by the Arrhenius equation.

$$\ln(K) = A + (-\frac{E_a}{R})\frac{1}{T} \qquad (2.15)$$

Slopes ($-E_a/R$) and intercepts (A) of these plots defined this equation for each transformation, and the resulting equations could be used for determining the rate constant at any temperature. Table 4.4 presents the activation energies obtained from the slope of Arrhenius plots.

Table 4.4: Activation energies for different transformations

Transformation	Activation energy
Inactivation of dormant spores, Sapru Model	62.93 (KCal/mol)
Inactivation of activated spores, Sapru Model	86.26 (Kcal.mol)
Activation of dormant spores, Sapru Model	84.90 (KCal/mol)
Activation of dormant spores, Shull Model	91.18 (Kcal/mol)
Activation of dormant spores, Rodriguez Model	91.05 (Kcal/mol)

Note that the activation energies for inactivation of activated spores are equal in the Sapru, Shull, Rodriguez, and Conventional, single-term models, and equal the inactivation rate constant for dormant spores in the Rodriguez model. Arrhenius equations for respective rate constants are also identical.

The effects of temperature on the rate constants of the Sapru, Shull and Rodriguez model were described by the following Arrhenius equations

$$\ln(K_{d2}) = 110.7 - \frac{43407.8}{T_a} \qquad (All\ Models) \qquad (4.1a)$$

$$\ln(K_{d1}) = 79.3 - \frac{31668.9}{T_a} \qquad (Sapru\ Model) \qquad (4.1b)$$

$$\ln(K_s) = 108.9 - \frac{42732.0}{T_s} \qquad (Sapru\ Model) \qquad (4.1c)$$

$$\ln(K_s) = 116.9 - \frac{45883.8}{T_s} \qquad (Shull\ Model) \qquad (4.1d)$$

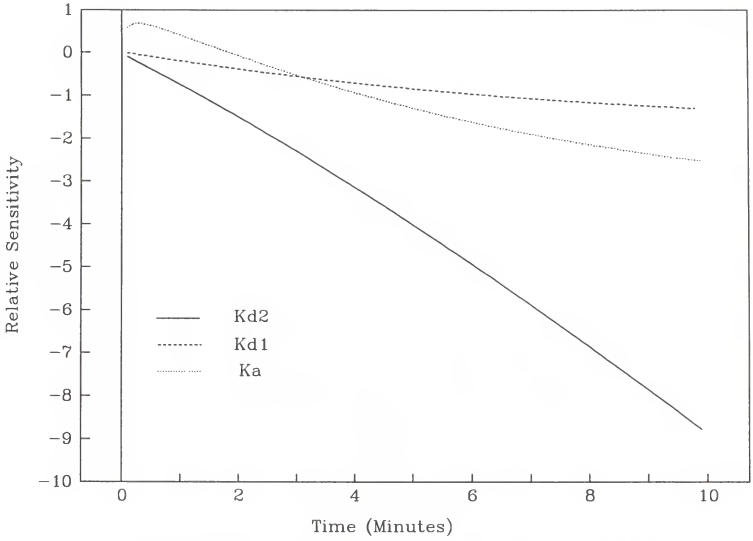
$$\ln(K_s) = 117.1 - \frac{45819.0}{T_s} \qquad (Rodriguez\ Model) \qquad (4.1e)$$

4.3 Relative Sensitivity of Sapru Model

Relative sensitivity of the Sapru model response, $N_2(t)$, was observed for 1% changes in the rate constants K_s, K_{d1}, and K_{d2}. Fig. 4.31 indicates those relative sensitivities at different times in an isothermal heating process (120°C). The response was least sensitive to inactivation rate constant K_{d1} and it was sensitive to activation rate constant K_s only early in a process. However, the response was very sensitive to inactivation rate constant K_{d2} as the process proceeded which indicated a need for care in estimating this parameter.

4.4 Validation of Models for Non-Isothermal Temperature Histories

The experimental responses of spore survivor population $N_2(t)$ to the temperature history of Fig. 4.32 are shown in Figs.4.33 and 4.34 along with responses predicted by the Sapru, Shull, Rodriguez and conventional, single-term models. Similar experimental and model simulated responses of spore survivor population to the temperature history of Fig. 4.35 are shown in Figs. 4.36 and 4.37. Figs. 4.33 and 4.36 are plotted on the semi-log scale, whereas Figs. 4.34 and 4.37 are plotted on the absolute scale. In both temperature histories, the Sapru model predicted the experimental data closely. Responses predicted by the Shull and Rodriguez models also followed the experimental data, but not as closely as the Sapru model.



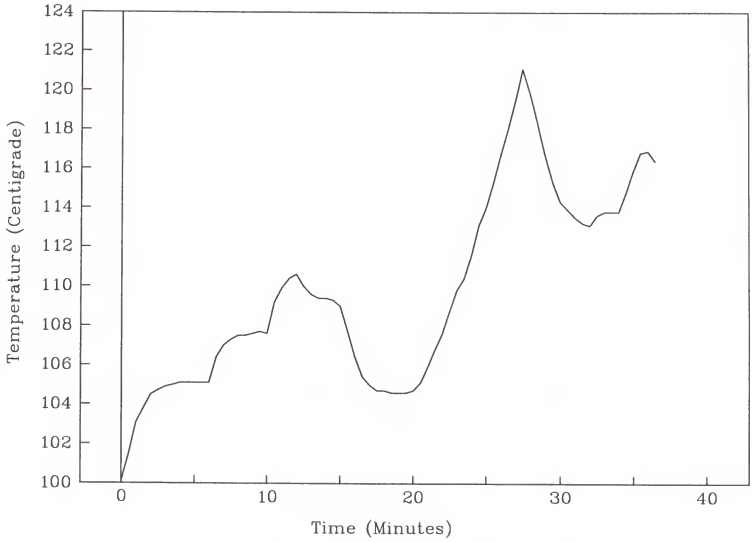
4.31 Relative sensitivity of the Sapru model response to its parameters

The assumption that all spores must be activated before being inactivated caused the Shull model to predict a higher number of survivors than the experimental data because the lack of dormant spore inactivation resulted in a greater number of activated spores overall. However, in an isolated instance (Figs. 4.33 and 4.34), the response predicted by the Shull model followed the experimental survivors better than the other models when subjected to the temperature history of Fig. 4.32. This was attributed to experimental aberration since, in all other cases, the Shull model, consistently over-estimated the number of survivors.

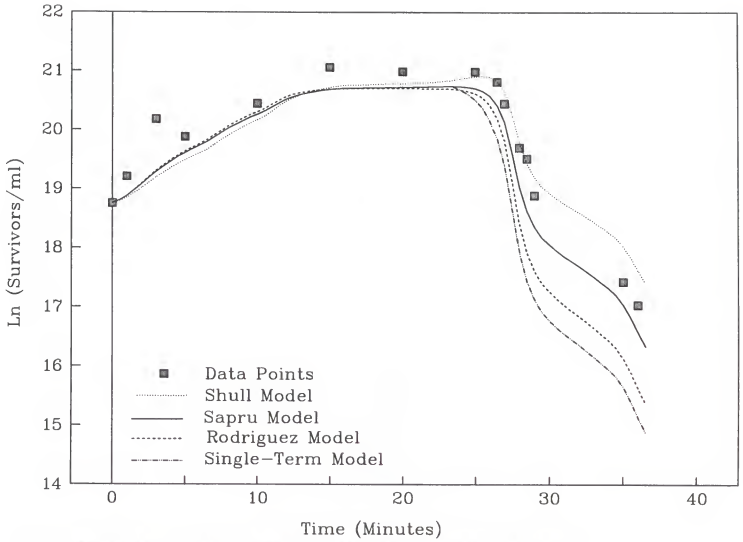
The assumption of identical inactivation rate constants for dormant and activated spores in the Rodriguez model caused higher inactivation of dormant spores and resulted in quick reduction of their number. Consequently, as the process advanced, a lesser number of spores remained for activation and the Rodriguez model consistently under estimated the number of survivors.

In Figs. 4.33 through 4.37 responses of the conventional, single-term model are also plotted and the shortcomings of this model are clearly evident. In spite of assuming the initial population for this model to be the maximum activated spores predicted by the Sapru model (Actual heat shock for 60 minutes at 100°C, resulted in a lower count), the response predicted by this model fell significantly below the experimental response. This was attributed to the fact that activation of dormant spores contributes to N_2 beyond the starting point in time of the single-term model. This contribution is over looked by the conventional, single-term model.

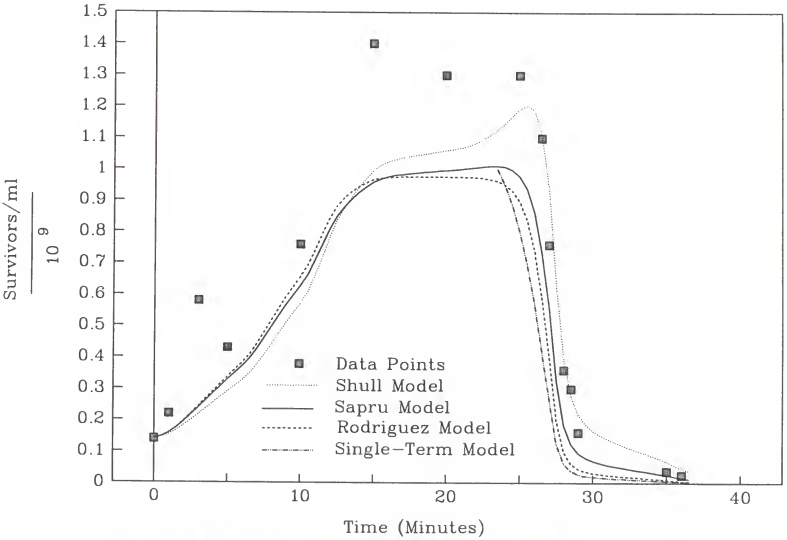
Figs. 4.38 and 4.39 compare experimental responses of heat shocked spores subjected to the dynamic temperature history of Fig. 4.35 with responses of the four models. Calculation of the response with the conventional, single-term model used initial spore count $N_0 = 7.22 \times 10^6$ (the count after heat shock for 60 minutes at 100°C), and for the Sapru,



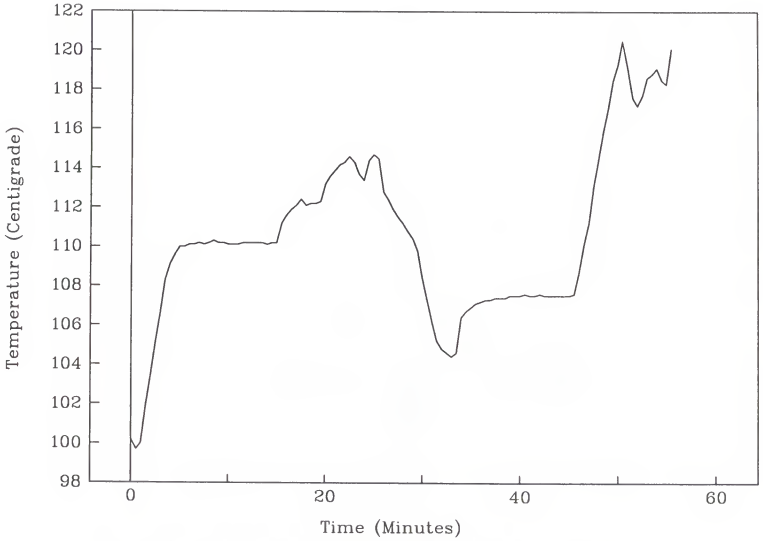
4.32 Dynamic temperature history for model validation (Appendix V-A)



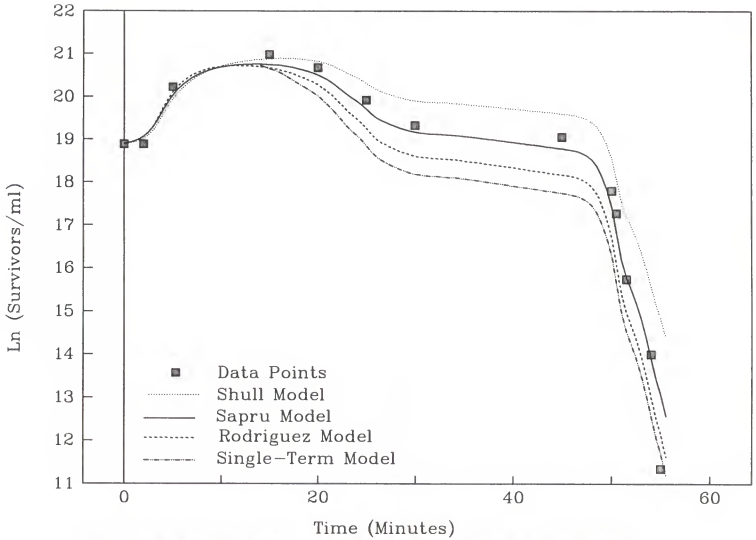
4.33 Actual survivors and predicted response to temperature history of Fig. 4.32 of all models



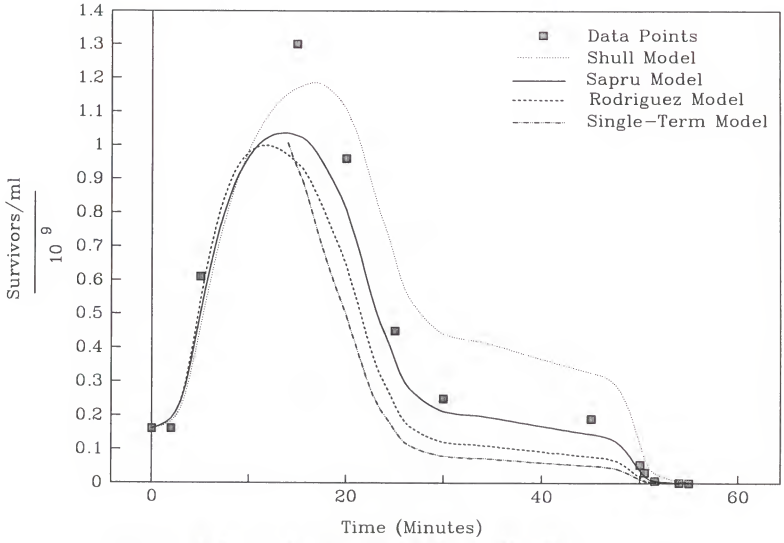
4.34 Actual survivors and predicted absolute response to temperature history of Fig. 4.32 of all models



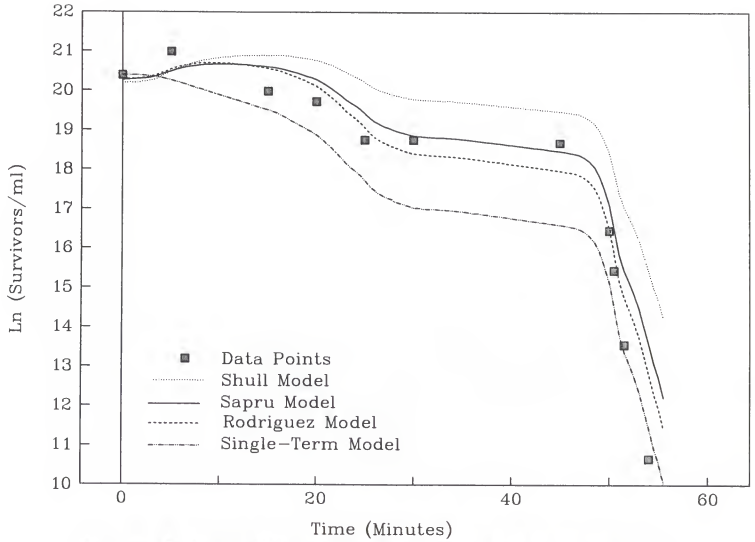
4.35 Dynamic temperature history for model validation (Appendix V-B)



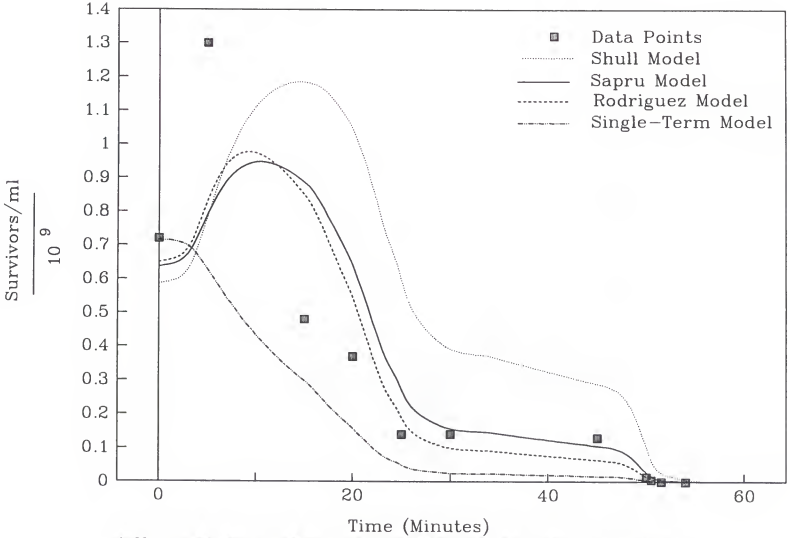
4.36 Actual survivors and predicted response to temperature history of Fig. 4.35 of all models



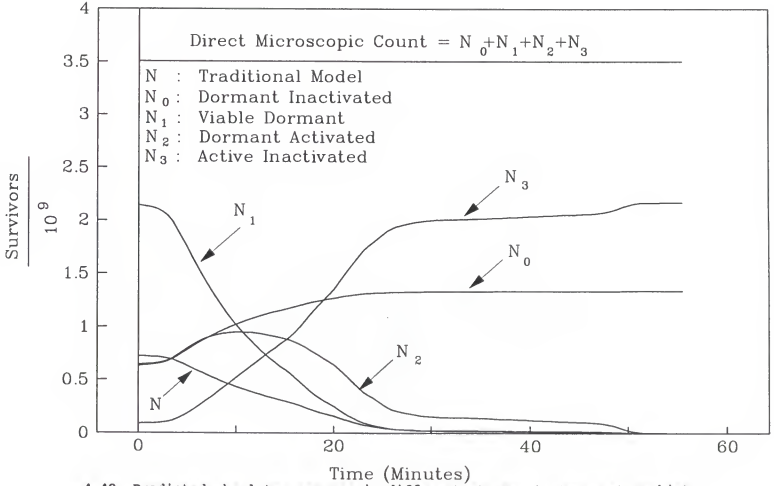
4.37 Actual survivors and predicted absolute response to temperature history of Fig. 4.35 of all models



4.38 Actual survivors and predicted response to temperature history of Fig. 4.35 of all models by heat shocked spores



4.39 Actual survivors and predicted absolute response to temperature history of Fig. 4.35 of all models by heat shocked spores



4.40 Predicted absolute response in different stores, to temperature history of Fig. 4.35 of Sapru and conventional models of heat shocked spores

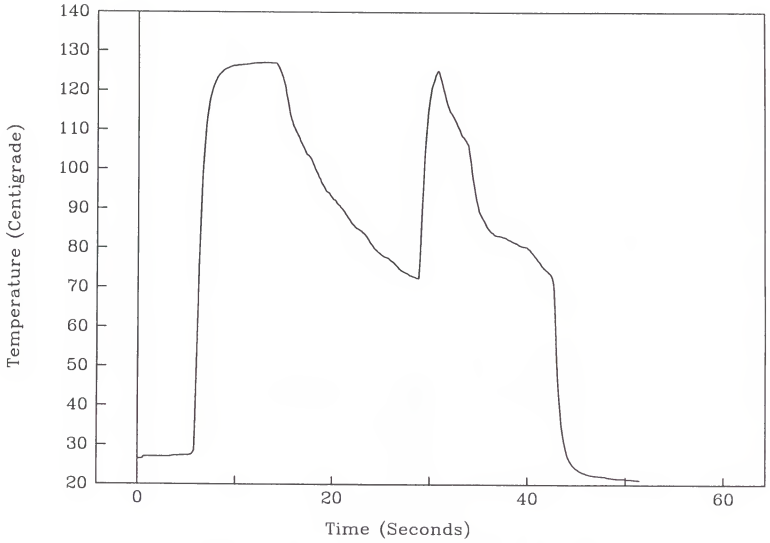
Shull and Rodriguez models used initial number of dormant spores N_{10} = $2.2x10^9$ and activated spores N_{20} = $6.4x10^8$ (Obtained by simulation of a uniform treatment at 100°C for 60 minutes). It is again evident that the Sapru, Shull and Rodriguez models predicted the survivor response more accurately, than the conventional, single-term model and that was attributable to the fact that activation of dormant spores contributes to N_2 beyond the starting point of the single-term model as was clearly shown (Fig. 4.40) by simulating changes of populations in different stores using the Sapru model. Although maximum spore counts were obtained within the first 10 minutes of the process, activation of dormant spores was not completed till much later in the process when population of dormant spores N_1 was reduced to zero.

4.5 Extrapolation of Rate Constants to UHT range

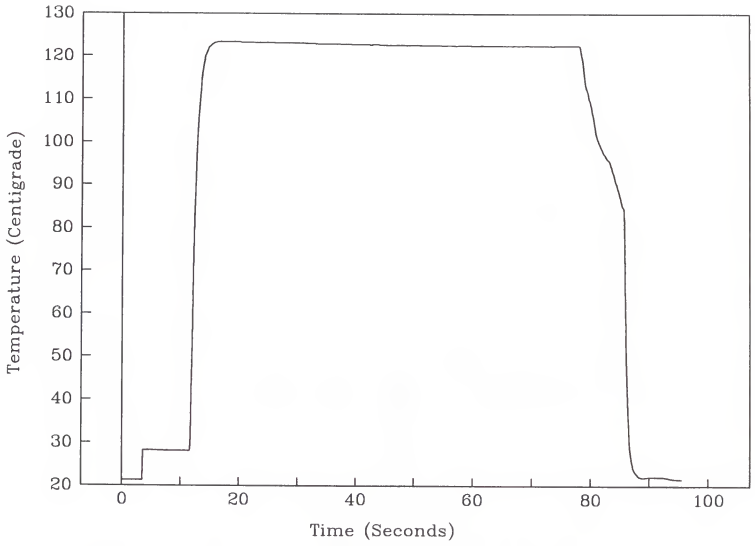
The Sapru model, using rate constants extrapolated by Arrhenius equations developed for rate constants in the sub-UHT range, successfully predicted survivors of spores subjected to ultra high temperature. The results from capillary tube, and steam reactor experiments are presented below.

4.5.1 Capillary tube experiments

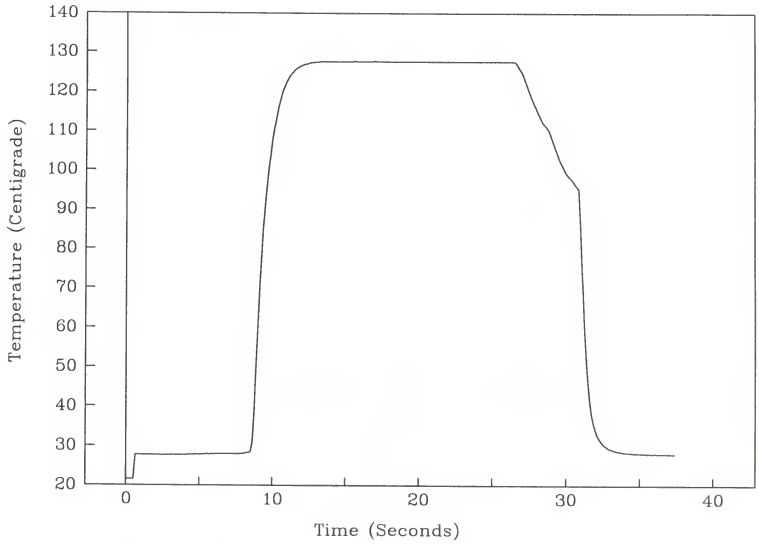
Fig. 4.41 through 4.51 depict UHT histories to which test spore suspensions were subjected in sealed capillary tubes. Fig. 4.52 is the scatter diagram of experimental and predicted number of survivors at the completion of each treatment (Table 4.5). Final survivors of temperature histories of Fig. 4.41 through 4.45 were the average counts of two identical samples. All others measurements were for single samples. Agreement between experimental and predicted numbers of final survivors was high, indicating that the Sapru model adequately predicted survivors of UHT treatments. Those findings also suggested that poor agreement of results from previous studies attempting to extrapolate rate constants to the UHT range using Arrhenius or TDT curves developed



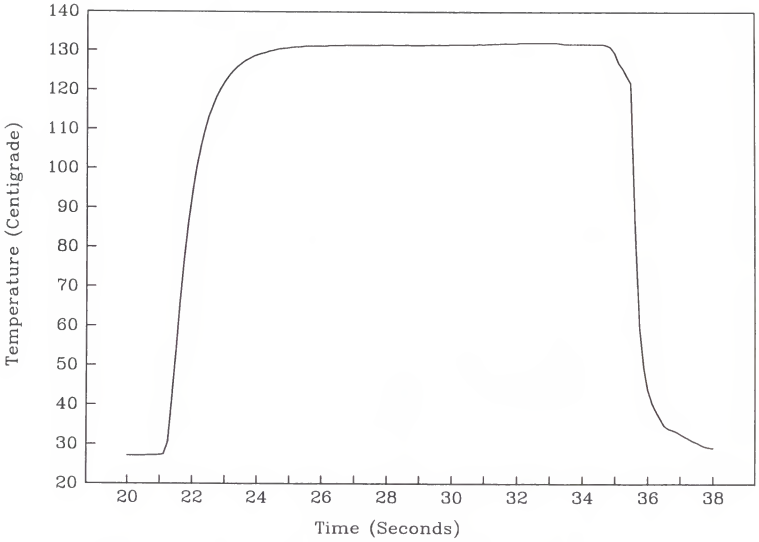
4.41 Sample UHT history (Appendix V-C1) in a capillary tube (i)



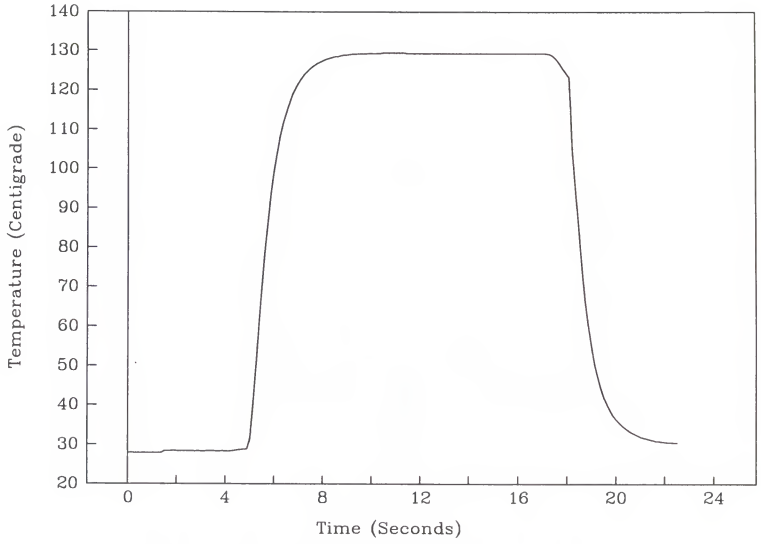
4.42 Sample UHT history (Appendix V-C2) in a capillary tube (ii)



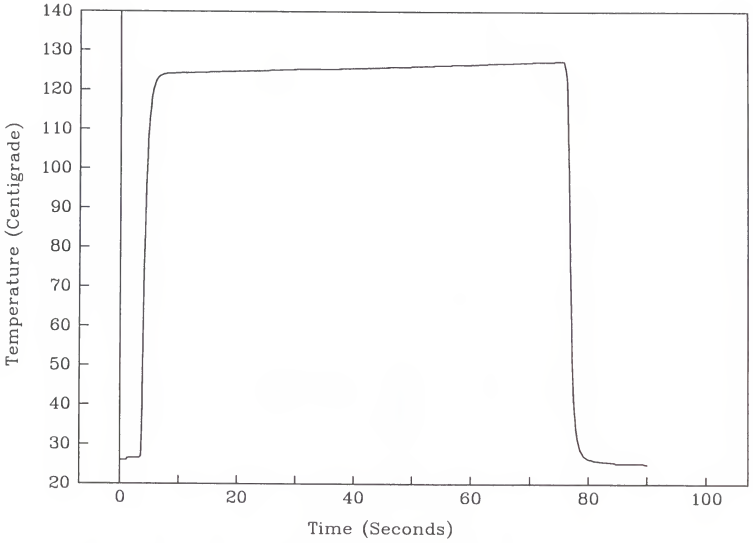
4.43 Sample UHT history (Appendix V-C3) in a capillary tube (iii)



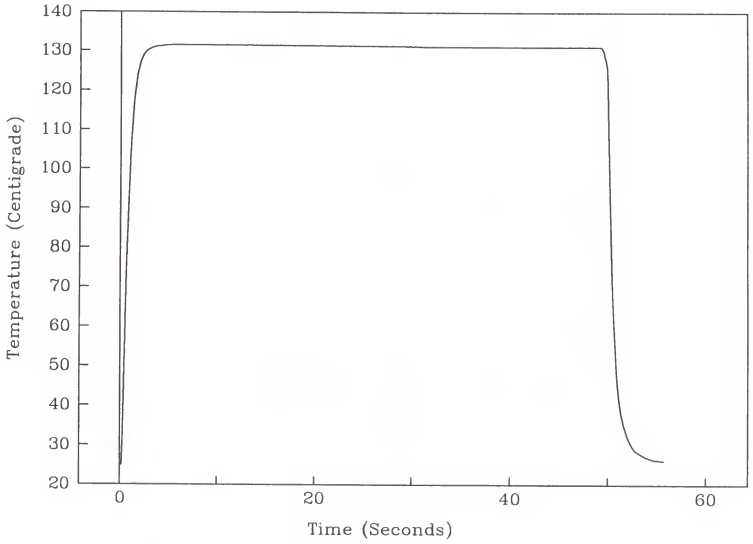
4.44 Sample UHT history (Appendix V-C4) in a capillary tube (iv)



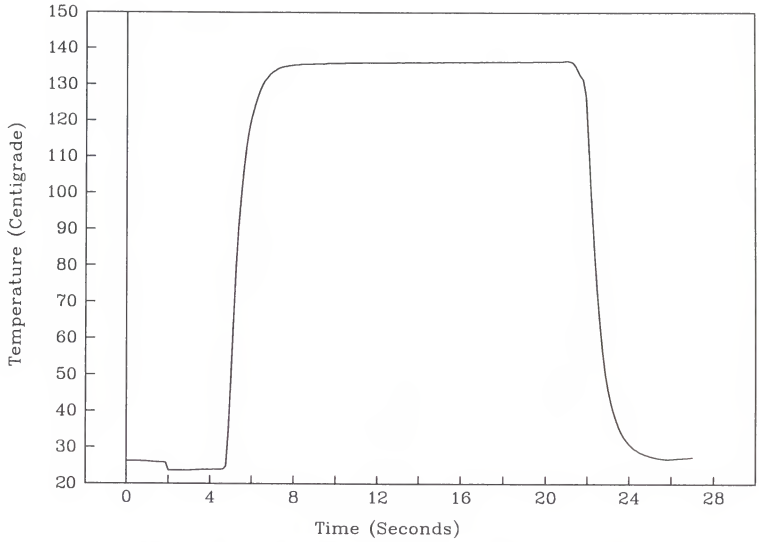
4.45 Sample UHT history (Appendix V-C5) in a capillary tube (v)



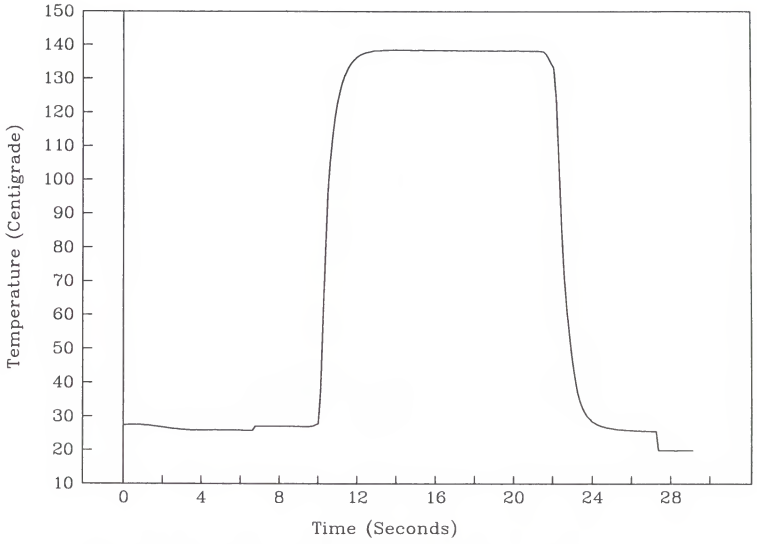
4.46 Sample UHT history (Appendix V-C6) in a capillary tube (vi)



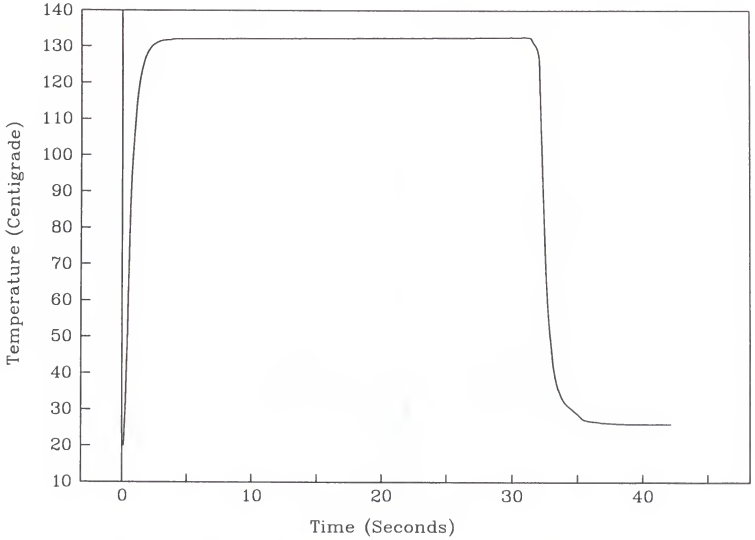
4.47 Sample UHT history (Appendix V-C7) in a capillary tube (vii)



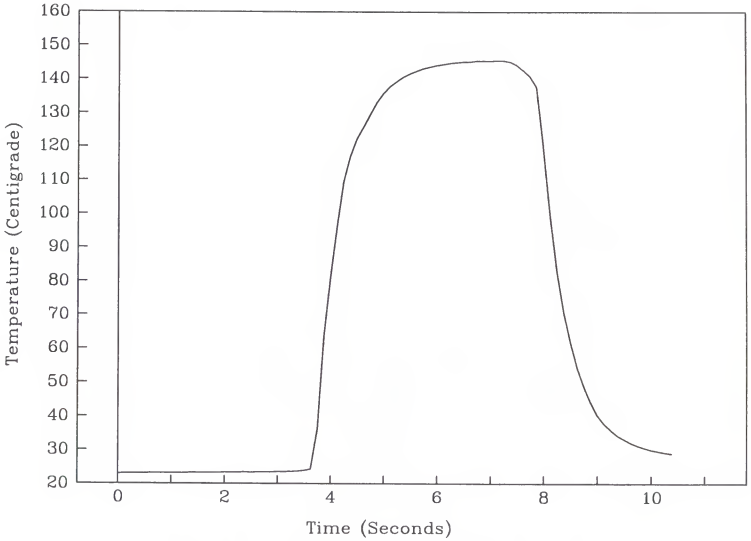
4.48 Sample UHT history (Appendix V-C8) in a capillary tube (viii)



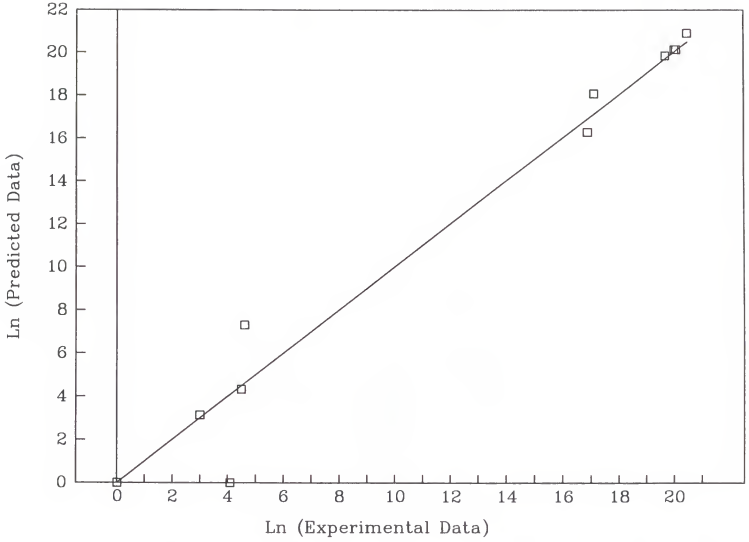
4.49 Sample UHT history (Appendix V-C9) in a capillary tube (ix)



4.50 Sample UHT history (Appendix V-C10) in a capillary tube (x)



4.51 Sample UHT history (Appendix V-C11) in a capillary tube (xi)



4.52 Scatter diagram for survivors of UHT histories of Figs. 4.41 through 4.51

at lower temperatures likely were due to the inadequacy of the single-term model, and not any fault of the Arrhenius temperature dependency.

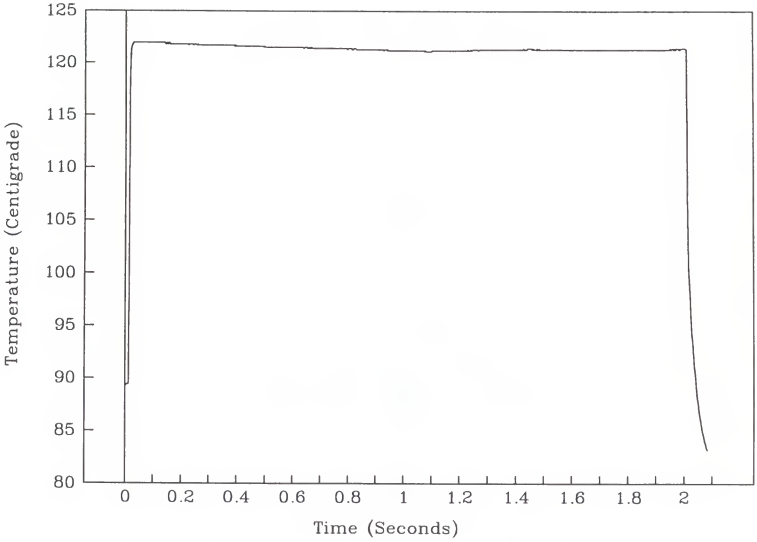
Table 4.5: Comparison of actual and predicted numbers of final survivors when test spore suspension in a sealed capillary tube was subjected to temperature histories of Figs. 4.41 through 4.51

Temperature history	Predicted Survivors/ml	Actual Survivors/ml	Ln(Predicted Surv)	Ln(Actual Surv)
Fig. 4.41	1.2×10^9	7.9×10^8	20.9	20.5
Fig. 4.42	4.2×10^8	3.7×10^8	19.9	19.7
Fig. 4.43	5.6×10^8	5.1×10^8	20.1	20.0
Fig. 4.44	7.2×10^7	2.8×10^7	18.1	17.1
Fig. 4.45	5.6×10^8	5.4×10^8	20.1	20.1
Fig. 4.46	1.2×10^7	2.2×10^7	16.3	16.9
Fig. 4.47	23	20	3.1	3.0
Fig. 4.48	76	90	4.3	4.5
Fig. 4.49	1	60	0	4.1
Fig. 4.50	1500	100	7.3	4.6
Fig. 4.51	0	0	-	-

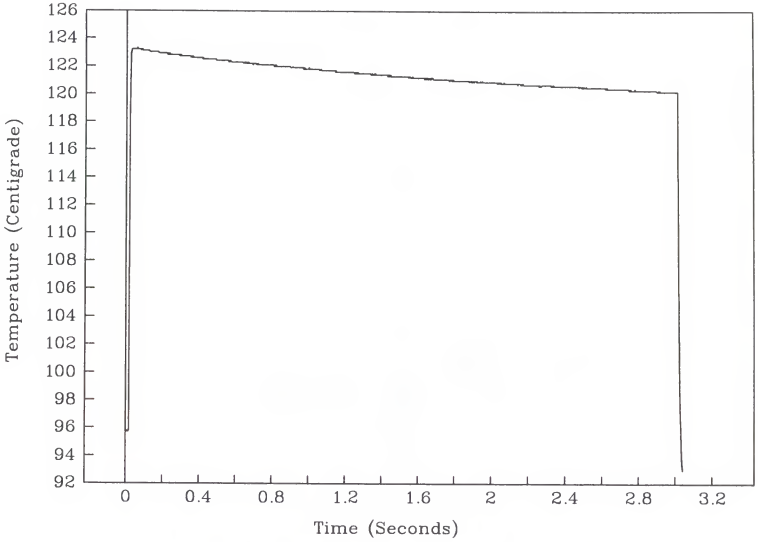
4.5.2 Steam reactor experiments

Fig. 4.53 through 4.57 depict UHT histories to which test spore suspensions were subjected in the steam reactor. Fig. 4.58 is the scatter diagram of experimental and predicted number of survivors (Table 4.6) at the end of the thermal process.

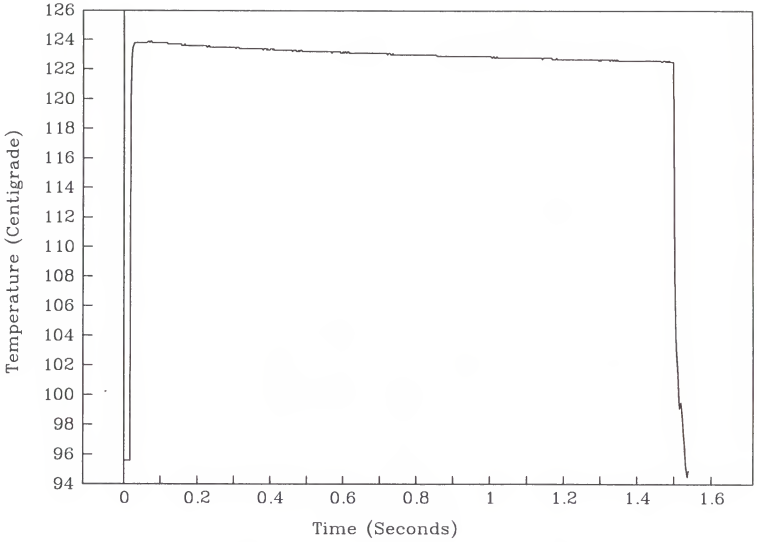
Poor agreement between actual and predicted numbers of final survivors for the temperature histories in these experiments was attributed to a number of factors pertaining to the apparatus. It was difficult to maintain integrity of the sample; about 10-20% of the sample was lost because of inability to recover it completely from the filter papers. Moreover, accumulation of condensate in the recovery tubes did not help the accuracy and attempts to account for that condensate by pre-weighing the recovery tubes did not significantly reduce the dispersion in the data or allow meaningful analysis.



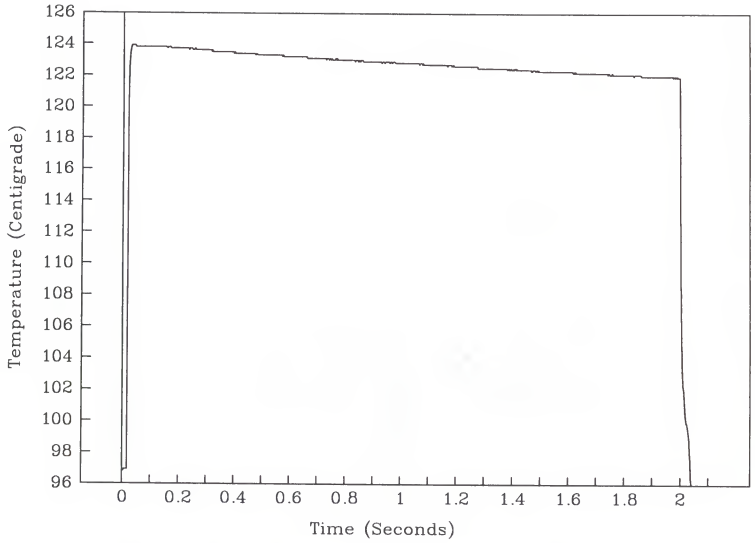
4.53 Sample UHT history (Appendix V-D1) in a steam reactor (i)



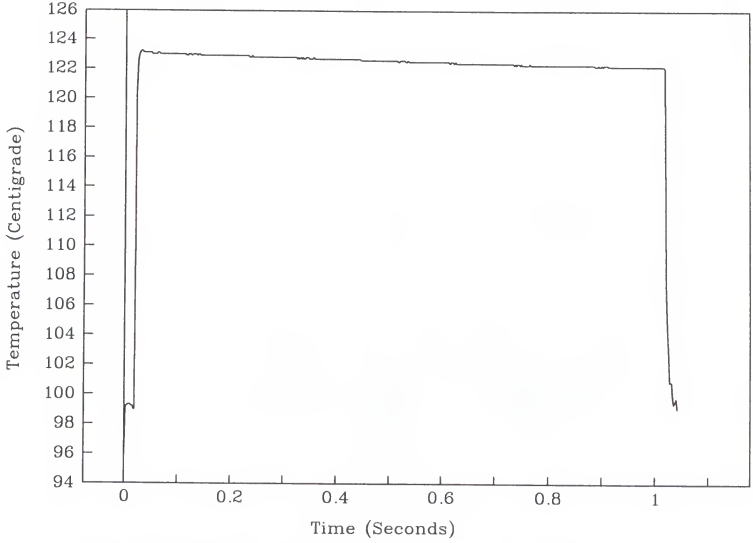
4.54 Sample UHT history (Appendix V-D2) in a steam reactor (ii)



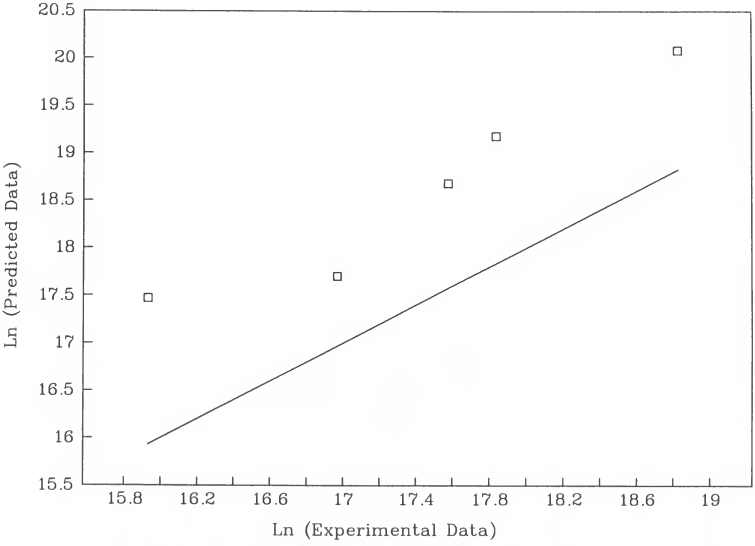
4.55 Sample UHT history (Appendix V-D3) in a steam reactor (iii)



4.56 Sample UHT history (Appendix V-D4) in a steam reactor (iv)



4.57 Sample UHT history (Appendix V-D5) in a steam reactor (v)



4.58 Scatter diagram for survivors of UHT histories of Figs. 4.52 through 4.57

Table 4.6: Comparison of actual and predicted numbers of final survivors when test spore suspension in a steam reactor was subjected to temperature histories of Figs. 4.53 through 4.57

Temperature history	Predicted Survivors/ml	Actual Survivors/ml	Ln(Predicted Surv)	Ln(Actual Surv)
Fig. 4.53	5.2×10^8	1.5×10^8	20.08	18.8
Fig. 4.54	2.1×10^8	5.6×10^7	19.18	17.8
Fig. 4.55	1.3×10^8	4.3×10^7	18.7	17.6
Fig. 4.56	4.8×10^7	2.3×10^7	17.7	17.0
Fig. 4.57	3.9×10^7	8.3×10^6	17.5	15.9

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

5.1 Conclusions

From this study the following conclusions were drawn:

1. A mathematical model (Sapru model) consisting of activation and two distinct inactivation transformations accurately predicted experimental survivor curves. Inclusion of activation in the model represented the basis for any increase of the survivor population early in a thermal process before its decline due to predominant inactivation. Furthermore, inclusion of activation precludes need for conventional heat shock to obtain a homogeneous population of activated spores prior to testing a thermal process.

2. Responses of the Shull, Rodriguez, and conventional, single-term models were compared with and showed the superiority of the Sapru model. The Sapru model adequately described isothermal survivor curves at the four experimental temperatures. Absence of the inactivation transformation for dormant spores in the Shull model resulted in a higher number of predicted survivors than the experimental data and the Sapru model. The assumption of identical inactivation rate constants for dormant and activated spores in the Rodriguez model resulted in higher inactivation of dormant spores than did the Sapru model and led to a lower number of activated spores in the declining phase than obtained experimentally and with the Sapru model.

3. Temperature dependency of the rate constants was well described by the Arrhenius equations in the sub-UHT range. Survivor curves predicted by the Sapru model, using the Arrhenius

equations to vary rate constants with temperature, showed close agreement with experimental data when spores were subjected to non-isothermal, dynamic heating processes in the sub-UHT range.

4. Extrapolation of rate constant values, to the UHT range by the Arrhenius equations established in the sub-UHT range permitted adequate predictions by the Sapru model of final numbers of survivors of dynamic heating processes in that range as well. Success of those extrapolations precluded the need for generating survivor curves at UHT to obtain rate constants for that range. Shortcomings of the conventional, single term model were clearly indicated and revealed the importance of the Sapru model to dependable design of sterilization processes in the UHT range.

5.2 Suggestions for Future Work

1. The Sapru model needs to be verified with other spore species and should be tested for real industrial processes involving food based substrates rather than buffers.

2. This model could be coupled with heat transfer models to evaluate lethalities in a product as well as to develop optimum time-temperature regimes for minimum nutritional and organoleptic damage to the product during pasteurization and sterilization processes.

3. The procedures used for development and validation of this model could be used in other relevant food engineering applications and should be applied to situations involving competing reactions, including enzyme kinetics and microbial growth.

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APPENDIX I
ANALYTICAL SOLUTION OF THE MATHEMATICAL MODEL

A: Response equation for dormant spores, N_1.

$$\frac{dN_1}{dt} = -(K_{d1} + K_a) N_1; \qquad N_1(0) = N_{10}$$

$$\frac{dN_1}{dt} + (K_{d1} + K_a) N_1 = 0$$

$$N_1(t) = Ce^{-(K_{d1}+K_a)t}; \qquad N_1(0) = N_{10} = C$$

$$N_1(t) = N_{10} e^{-(K_{d1}+K_a)t}$$

B: Response equation for activated spores, N_2.

$$\frac{dN_2}{dt} = K_a N_1 - K_{d2} N_2; \qquad N_2(0) = N_{20}$$

$$\frac{dN_2}{dt} + K_{d2} N_2 = K_a N_{10} e^{-(K_{d1}+K_a)t}$$

Particular solution: $N_{2p} = Ae^{-(K_{d1}+K_a)t}$

$$-(K_{d1} + K_a) Ae^{-(K_{d1}+K_a)t} + K_{d2} Ae^{-(K_{d1}+K_a)t} = K_a N_{10} e^{-(K_{d1}+K_a)t}$$

$$A = N_{10} \frac{K_a}{K_{d2} - (K_{d1} + K_a)}$$

$$N_{2p} = N_{10} \frac{K_a}{K_{d2} - (K_{d1} + K_a)} e^{-(K_{d1}+K_a)t}$$

Homogeneous solution: $N_{2h}=Ce^{-K_{d2}t}$

$$N_2(t)=Ce^{-K_{d2}t}+N_{10}\frac{K_a}{K_{d2}-(K_{d1}+K_a)}e^{-(K_{d1}+K_a)t}$$

$$N_2(0)=N_{20}=C+N_{10}\frac{K_a}{K_{d2}-(K_{d1}+K_a)} \quad ; \qquad C=N_{20}-N_{10}\frac{K_a}{K_{d2}-(K_{d1}+K_a)}$$

$$N_2(t)=(N_{20}-N_{10}\frac{K_a}{K_{d2}-(K_{d1}+K_a)})e^{-K_{d2}t}+N_{10}\frac{K_a}{K_{d2}-(K_{d1}+K_a)}e^{-(K_{d1}+K_a)t}$$

C: Response equation for inactivated dormant spores, N_0.

$$\frac{dN_0}{dt}=K_{d1}N_1 \qquad N_0(0)=N_{00}$$

$$\int_0^t dN_0=\int_0^t K_{d1}N_1\,dt=\int_0^t K_{d1}N_{10}e^{-(K_{d1}+K_a)t}dt$$

$$N_0(t)=N_{00}+N_{10}\frac{K_{d1}}{K_{d1}+K_a}(1-e^{-(K_{d1}+K_a)t})$$

D: Response equation for inactivated active spores, N_3.

$$\frac{dN_3}{dt}=N_2K_{d2} \quad ; \qquad N_3(0)=N_{30}$$

$$N_3(t)=N_{30}+K_{d2}\int_0^t N_2\,dt$$

$$N_3(t)=N_{30}+N_{20}K_{d2}\int_0^t e^{-K_{d2}t}dt+N_{10}\frac{K_{d2}K_a}{K_a+K_{d1}-K_{d2}}\int_0^t e^{-K_{d2}t}dt-N_{10}\frac{K_{d2}K_a}{K_a+K_{d1}-K_{d2}}\int_0^t e^{-(K_{d1}+K_a)t}dt$$

$$N_3(t)=N_{30}+(N_{20}+N_{10}\frac{K_a}{K_a+K_{d1}-K_{d2}})(1-e^{-K_{d2}t})-N_{10}\frac{K_{d2}K_a}{(K_a+K_{d1}-K_{d2})(K_{d1}+K_a)}(1-e^{-(K_{d1}+K_a)t})$$

E: Response equation for N_2, when $K_{d2}=K_{d1}+K_a$.

$$\frac{dN_2}{dt}=K_aN_1-K_{d2}N_2 \quad \Rightarrow \quad \frac{d^2N_2}{dt^2}=K_a\frac{dN_1}{dt}-K_{d2}\frac{dN_2}{dt}$$

$$\frac{d^2N_2}{dt^2}=K_a\left(-\left(K_{d1}+K_a\right)N_1\right)-K_{d2}\frac{dN_2}{dt}$$

Since $N_1=\frac{1}{K_a}\left(\frac{dN_2}{dt}+K_{d2}N_2\right)$

$$\frac{d^2N_2}{dt^2}+\frac{dN_2}{dt}\left(K_{d1}+K_a+K_{d2}\right)+K_{d2}\left(K_{d1}+K_a\right)N_2=0$$

Since $K_{d2}=K_{d1}+K_a$ $\Rightarrow$ *Characteristic quation:* $\lambda^2+2\lambda K_{d2}+K_{d2}^2$

Roots: $\lambda_1=\lambda_2=-K_{d2}$

$$N_2(t)=C_1e^{-K_{d2}t}+C_2te^{-K_{d2}t}$$

$$\frac{dN_2}{dt}=-K_{d2}C_1e^{-K_{d2}t}-C_2K_{d2}te^{-K_{d2}t}+C_2e^{-K_{d2}t}$$

$$N_2(0)=N_{20}=C_1+0 \quad \Rightarrow \quad C_1=N_{20}$$

$$\frac{dN_2}{dt}=K_aN_1-K_{d2}N_2$$

$$K_aN_{10}-K_{d2}N_{20}=-K_{d2}N_{20}+C_2 \quad \Rightarrow \quad C_2=K_aN_{10}$$

$$N_2(t)=N_{20}e^{-K_{d2}t}+N_{10}tK_ae^{-K_{d2}t}$$

APPENDIX II
SURVIVOR DATA FOR BACILLUS STEAROTHERMOPHILUS

A. Survivor data at 105°C

Set 1:		Set 2:		Set 3:	
Time (Minutes)	Survivors	Time (Minutes)	Survivors	Time (Minutes)	Survivors
0	2.7E+08	0	1.4E+08	0	1.7E+08
1	3.7E+08	1	2.5E+08	1	3.0E+08
2.5	4.9E+08	2.5	3.0E+08	2.5	3.9E+08
5	7.6E+08	5	5.1E+08	5	5.4E+08
10	7.6E+08	10	3.9E+08	10	6.2E+08
15	9.7E+08	15	6.9E+08	15	7.1E+08
25	1.3E+09	30	7.2E+08	25	8.2E+08
35	1.1E+09	45	6.4E+08	30	8.6E+08
45	1.4E+09	60	7.6E+08	35	8.9E+08
60	1.0E+09	90	4.5E+08	45	8.2E+08
120	9.1E+08	120	4.5E+08	60	9.1E+08
170	2.2E+08	150	1.8E+08	90	6.8E+08
300	32700000	180	1.7E+08	120	4.0E+08
480	90000	300	51000000	150	2.8E+08
		390	14000000	180	2.5E+08
		480	5800000	300	64000000
				390	8200000
				480	132000

B. Survivor data at 110°C

Set 1:		Set 2:		Set 3:	
Time (Minutes)	Survivors	Time (Minutes)	Survivors	Time (Minutes)	Survivors
0	1.4E+08	0	1.7E+08	0	2.2E+08
0.5	4.6E+08	0.5	4.2E+08	0.5	3.9E+08
1	4.9E+08	1	5.1E+08	1	4.5E+08
1.5	6.8E+08	2	6.1E+08	2	4.6E+08
2	8.5E+08	4	1.2E+09	4	6.2E+08
4	1.2E+09	6	9.6E+08	6	6.9E+08
6	1.2E+09	10	1.2E+09	10	8.2E+08
10	1.1E+09	15	1.2E+09	15	8.0E+08
15	2.2E+08	20	1.2E+09	20	9.7E+08
20	1.2E+09	30	8.9E+08	30	5.7E+08
30	6.6E+08	40	4.8E+08	40	2.5E+08
40	3.6E+08	60	2.5E+08	60	1.5E+08
60	1.9E+08	90	70000000	90	23000000
90	48000000	120	8700000	120	1000000
120	5970000	180	18000	180	3600
180	5430				

C. Survivor data at 115°C

Set 1:		Set 2:		Set 3:	
Time (Minutes)	Survivors	Time (Minutes)	Survivors	Time (Minutes)	Survivors
0	1.4E+08	0	1.7E+08	0	2.2E+08
0.25	6.5E+08	0.25	5.1E+08	0.25	4.5E+08
0.5	8.9E+08	0.5	7.3E+08	0.5	8.7E+08
0.75	7.6E+08	1	1.1E+09	1	1.0E+09
1	7.9E+08	2	1.2E+09	2	1.3E+09
1.5	1.4E+09	3	1.1E+09	3	1.3E+09
2	1.1E+09	5	1.4E+09	5	9.2E+08
2.5	1.0E+09	10	5.0E+08	10	3.3E+08
3	1.7E+09	15	1.7E+08	15	1.1E+08
15	1.2E+09	20	76000000	20	27000000
45	14000	30	6800000	30	840000
		45	120000	45	1700
				60	20

D. Survivor data at 120°C

Set 1:		Set 2:		Set 3:	
Time (Minutes)	Survivors	Time (Minutes)	Survivors	Time (Minutes)	Survivors
0	1.4E+08	0	1.7E+08	0	2.2E+08
0.08	4.3E+08	0.08	5.9E+08	0.08	3.4E+08
0.17	1.0E+09	0.17	6.9E+08	0.17	4.8E+08
0.33	9.8E+08	0.33	1.2E+09	0.33	8.3E+08
0.67	7.4E+08	0.67	1.3E+09	0.67	1.4E+09
1	6.5E+08	1	1.1E+09	1	1.1E+09
1.33	4.1E+08	1.33	1.1E+09	1.33	8.8E+08
1.67	3.7E+08	1.67	7.1E+08	1.67	6.4E+08
2	2.4E+08	2	7.2E+08	2	6.1E+08
3	1.2E+08	4	76000000	4	50000000
4	46000000	6	7700000	6	6800000
6	7300000	8	350000	8	580000
8	120000	10	3620	10	1760
10	4000	12	260		
		14	40		

E. Survivors of dynamic temperature histories

Temperature history of Appendix V-A, scan rate=1/4th sec.

Time (Minutes)	Survivors	
0	1.4E+08	
3	5.8E+08	
5	4.3E+08	
10	7.6E+08	
15	1.4E+09	
20	1.3E+09	
25	1.3E+09	
26.5	1.1E+09	
27	7.6E+08	
27.5	7.9E+08	(broke)
28	3.6E+08	
28.5	3.0E+08	
29	1.6E+08	
35	37000000	
36	25000000	
38	4000000	(no temperature history)

Temperature history of Appendix V-B, scan rate, dt=1/4th sec.

Heat Activated For 60 min. at 100°C		Untreated	
Time (Minutes)	Survivors	Time (Minutes)	Survivors
0 (60)	7.2E+08	0	1.6E+08
5	1.3E+09	2	1.6E+08
15	4.8E+08	5	6.1E+08
20	3.7E+08	15	1.3E+09
25	1.4E+08	20	9.6E+08
30	1.4E+08	25	4.5E+08
45	1.3E+08	30	2.5E+08
50	14000000	45	1.9E+08
50.5	5100000	50	54000000
51.5	770000	50.5	32000000
54 (114)	43000	51.5	6900000
		54	1200000
		55	85000

F. Survivors of UHT histories Capillary Experiments
(Scan rate=¼th sec.)

History of	Survivors Sample #1	Sample #2
Appendix V-C1	7.7E+08	8.1E+08
Appendix V-C2	3.4E+08	3.9E+08
Appendix V-C3	5.0E+08	5.1E+08
Appendix V-C4	18000000	37000000
Appendix V-C5	5.3E+08	5.5E+08
Appendix V-C6	22000000	
Appendix V-C7	20	
Appendix V-C8	90	
Appendix V-C9	60	
Appendix V-C10	100	
Appendix V-C11	0	

G. Survivors of UHT histories (reactor experiments)
(Scan rate=1/8th sec.)

History of	Survivors
Appendix-D1	56000000
Appendix-D2	8300000
Appendix-D3	43000000
Appendix-D4	23400000
Appendix-D5	1.5E+08

APPENDIX III
PASCAL PROGRAMS

A. Pascal program for initial estimates of model parameters

```
Program Residual;

Uses
  Dos, Crt, Printer, GDriver, GKernel, GWindow, GShell;

Const  N10=3.23e9; MaxDat=20;

Type
  Arr2 = ARRAY [1..MaxDat,1..2] of real;
  Arr1 = ARRAY [1..MaxDat] of real;

Var
  Data: Arr2;
  Sd: Arr1;
  Ndata,Max,Rp:Integer;
  I,J,Iter:Integer;
  Ioerr:Boolean;
  X1,X2,X3,X4:Real;

Procedure iocheck;
var
   iocode:integer;
procedure error(msg:string);
var
   ch:char;
begin
    gotoxy(1,24);clreol;
    write('G,msg,' hit any key to return');
    ch:=readkey;
end;
begin
   iocode:=ioresult;
   ioerr:=(iocode<> 0);
    if ioerr then
    begin
      case iocode of
         $02 : error('file not found');
         $03 : error('path not found');
         $04 : error('file not open');
         $10 : error('error in numeric format');
         $20 : error('operation not allowed on logical device');
         $21 : error('not allowed in direct mode');
         $22 : error('assign to standard files not allowed');
         $90 : error('record length mismatch');
         $91 : error('seek beyond end of line');
         $99 : error('unexpected end of file');
         $F0 : error('disk write error');
         $F1 : error('directory is full');
```

```
        $F2 : error('file size overflow');
        $F3 : error('too many open files');
        $FF : error('file disappeared');
      else
        error('unknown I/O error:  ');
        write(iocode:3)
      end
    end
end;

Procedure ReadData(Var data:Arr2; Var Sd:Arr1;  Var Ndata:Integer);
Var
  DataFile:Text;
  Filename:String;
begin
 repeat
{$I-}
  repeat
    clrscr;
    gotoxy(1,10);
    write('ENTER SURVIVOR FILENAME OR  Q  TO EXIT: ' );
    readln(filename);
  until (filename<>'');
    if (filename='q') or (filename='Q') then halt;
    assign(datafile,filename);
    reset(datafile);
    iocheck;
until ioerr=false;
    i:=0;
    while not eof(datafile) do
     begin
       i:=i+1;
       readln(datafile,data[i,1],data[i,2],Sd[i]);
     end;
  ndata:=i;
  close(datafile);
{$I+}
clrscr;
end;

Procedure Plot(X1,X2,X3,X4:real);
var
    Reg,lndata,lnreg:plotarray;
    i:integer;
    A,B,C,D,Sum:Real;
Begin
{Regression Equation X1*exp-(X2*t)-X3*exp-(X4*t)}
  For i:=1 to ndata do
    begin
      B:=X2*data[i,1];
      D:=X4*data[i,1];
      A:=X1*Exp(-B) ;
      C:=X3*Exp(-D) ;
      Reg[i,1]:=data[i,1];
      Reg[i,2]:=A-C;
      if Reg[i,2]<=0 then
       begin
        clrscr;
        gotoxy(1,10);
        write('Negative Regression ',i,' ',Reg[i,2],' hit <return> to
continue');
        readln;
```

```
        Reg[i,2]:=1;
        end;
    end;
    for i:=1 to ndata do
     begin
       lndata[i,1]:=data[i,1];
       lndata[i,2]:=ln(data[i,2])/2.303;
       lnreg[i,1]:=reg[i,1];
       lnreg[i,2]:=ln(reg[i,2])/2.303;
     end;
   entergraphic;
   SetLineStyle(0);
   setbackgroundcolor(1) ;
   setforegroundcolor(15);
   definewindow(1,10,10,trunc(xmaxglb-10),trunc(ymaxglb*3/4));
   defineheader(1,'REGRESSION EQUATION PLOT');
   definewindow(2,0,trunc(ymaxglb*3/4),xmaxglb,ymaxglb);
   setheaderon;
   FindWorld(1,lndata,ndata,1.2,1.2);
   SelectWindow(1);
   drawborder;
   drawAxis(5, -5, 1, 0, 0, 0, 0, 0, false);
   SetLineStyle(1);
   DrawPolygon(lndata,1,-ndata, 3, 3, 0); {clipping on}
   SetLineStyle(0);
   ResetAxis;
   DrawPolygon(lnReg,1,-ndata, 0, 0, 0);{clipping on}
   selectwindow(2);
   gotoxy(20,20);
   write('   ------ Time (Minutes) ------->');
   gotoxy(8,3);
   write('L');
   gotoxy(8,4);
   write('o');
   gotoxy(8,5);
   write('g');
   gotoxy(8,7);
   write('S');
   gotoxy(8,8);
   write('u');
   gotoxy(8,9);
   write('r');
   gotoxy(8,10);
   write('v');
   gotoxy(8,11);
   write('i');
   gotoxy(8,12);
   write('v');
   gotoxy(8,13);
   write('o');
   gotoxy(8,14);
   write('r');
   gotoxy(8,15);
   write('s');
   gotoxy(12,22);

writeln('Model=',X1:5,'*Exp(-',X2:5:4,'*t)-',X3:5,'*Exp(-',X4:5:4,'*t)')
;
   gotoxy(12,23);
   writeln('kd1=',X4-X3*(X4-X2)/N10:5:3,' kd2=',X2:5:3,'
ka=',X3*(X4-X2)/N10:5:3);
   gotoxy(12,25);
```

```
   writeln('HIT ANY KEY TO CONTINUE ');
   readln;
   leavegraphic;
end;

Procedure Residual(Var X1,X2,X3,X4:real; var max:Integer);
var
   i,nmax:integer;
   lin:PlotArray;
   a1,b1,a2,b2:real;
Procedure LinFit(Var A,B:real;dat:plotarray;n:integer);
var
      i:integer;
     StdX,StdY,Sum_X, Sum_Y, Sum_XY, Sum_X2, Sum_Y2,
     Xi, Yi, SXY, SXX, SYY : real;
begin
     Sum_X:=0.0;
     Sum_Y:=0.0;
     Sum_XY:=0.0;
     Sum_X2:=0.0;
     Sum_Y2:=0.0;
    for i:=1 to n do
    begin
     xi:=dat[i,1];
     yi:=dat[i,2];
     Sum_X:=Sum_X+xi;
       Sum_Y:=Sum_Y+yi;
     Sum_XY:=Sum_XY+Xi*Yi;
     Sum_X2:=Sum_X2+Xi*Xi;
     Sum_Y2:=Sum_Y2+Yi*Yi;
    end;
    SXX:=Sum_X2-Sum_X*Sum_X/N;
    SXY:=Sum_XY-Sum_X*Sum_Y/N;
    SYY:=Sum_Y2-Sum_Y*Sum_Y/N;
    B:=SXY/SXX;
    A:=((Sum_X2*Sum_Y-Sum_X*Sum_XY)/N)/SXX;
end;

begin
     nmax:=1;
     for i:=1 to ndata-1 do
     begin
      if (data[i,2]<data[i+1,2]) then nmax:=i+1;
     end;
     max:=nmax;
     nmax:=nmax+Rp;
     for i:=1 to ndata-nmax+1 do
      begin
       lin[i,1]:=data[nmax-1+i,1];
       lin[i,2]:=ln(data[nmax-1+i,2]);
      end;
        linfit(a1,b1,lin,ndata-nmax+1);
        for i:=1 to ndata do
        begin
          lin[i,2]:=abs(data[i,2]-exp(a1+b1*data[i,1]));
        end;
     for i :=1 to nmax do
      begin
        lin[i,1]:=data[i,1];
        lin[i,2]:=ln(lin[i,2]); end;
        linfit(a2,b2,lin,nmax);
        X1:=exp(a1);
```

```
        X2:=-b1;
        X3:=exp(a2);
        X4:=-b2; end;

Begin
Initgraphic;
Leavegraphic;
Repeat
Rp:=2;
  ReadData(data,sd,ndata);
 Repeat
  Residual(X1,X2,X3,X4,max);
  Plot(X1,X2,X3,X4);
  {$I-} repeat
      clrscr;
      GotoXY(1,10);
      Write('Enter new trial regression point between ',trunc(-max/2):2,'
and ',
      trunc((ndata-max)/2):1,', To exit enter 10: ');
      Readln(Rp);
      iocheck;
    until ioerr=false;
    {$I+} Until Rp=10;
  Until false;
End.
```

B. SAS nonlinear regression procedure for estimation of rate constants

```
Title 'non linear regression Cap105.com'
data nonlin;
infile 'cap105.com';
Input t s;
n10=3.3E9;
n20=2.3E8;
kd2=0.017;
Proc nlin;
parms ka=1.6168 kdl=0.444;
Model s=(n20+n10*ka/(kdl+ka-kd2))*exp(-kd2*t)-
        n10*ka/(kdl+ka-kd2)*exp(-(kdl+ka)*t);
der.Ka = n10/((kdl+ka-kd2)*(kdl+ka-kd2))*(((kdl+ka-kd2)-ka)*exp(-kd2*t)+
         (t*ka*(kdl+ka-kd2)+ka-(kdl+ka-kd2))*exp(-(kdl+ka)*t));
der.Kdl= ka*n10/((kdl+ka-kd2)*(kdl+ka-kd2))*((t*(kdl+ka-kd2)+1)*
         exp(-(kdl+ka)*t)-exp(-kd2*t));
proc print;
run;
```

C. Pascal program for sensitivity analysis of the model

```
Program Sensitivity;

Const N10=3.2E9; N20=2.3E8;
Var
  relsenkd2,relsenka,relsenkdl,dt,Ka,Kdl,Kd2,T:Real;
  Fout:Text;
  i:Integer;

Function Surv(Kd1,Ka,Kd2,T:Real):Real;
Begin

Surv:=(N20+N10*KA/(KD1+KA-KD2))*EXP(-KD2*T)-N10*KA/(KD1+KA-KD2)*EXP(-(KD
1+KA)*T);
End;

Begin
   Kd2:=1.35;
   Ka:=1.1671;
   Kd1:=0.43;
   dt:=0.1;
   Assign(Fout,'D:\vimal\lotus\research\sensitiv.prn');
   Rewrite(Fout);
   For i:= 1 to 100 do
   begin
    t:=i*dt;
relsenKd2:=(Surv(Kd1,Ka,1.01*Kd2,t)-Surv(Kd1,Ka,Kd2,t))/
Surv(Kd1,Ka,Kd2,t)/0.01;
relsenKa:=(Surv(Kd1,1.01*Ka,Kd2,t)-Surv(Kd1,Ka,Kd2,t))/
Surv(Kd1,Ka,Kd2,t)/0.01;
relsenKd1:=(Surv(1.01*Kd1,Ka,Kd2,t)-Surv(Kd1,Ka,Kd2,t))/
Surv(Kd1,Ka,Kd2,t)/0.01;
    writeln(fout,t,relsenkd2,relsenka,relsenkd1);
   end;
End.
```

D. Pascal program for prediction of survivors subjected to dynamic temperature history using new model

```
Program Survivor;

Var
 NO, N00, N10, N20, N30, NN, N0, N1, N2, N3,
 KKa, KKd1, KKd2, KK,
 Temp, TempOld,t, Dt, AbsTemp, AvTemp :Real;
 i:  Integer;
 fin, fout :Text;
 notin:Boolean;
Function Kd2(AvTemp:real):real;
Var
   AbsTemp          :real;
begin
   AbsTemp:=AvTemp+273.15;
   Kd2:=exp( 110.6511-43407.8/AbsTemp);
end;{kd2}

Function Ka(AvTemp:real):real;
var
   AbsTemp          :real;
begin
   AbsTemp:=AvTemp+273.15;
   Ka:=exp( 108.8545-42732/AbsTemp);
end;{ka}

Function Kd1(AvTemp:real):real;
var
   AbsTemp          :real;
begin
   AbsTemp:=AvTemp+273.15;
   Kd1:=exp( 79.2922-31668.9/AbsTemp);
end;{kd1}

begin
   assign(fin,'c:\vimal\acapnon.prn');
   reset(fin);
   assign(fout,'d:\vimal\out.prn');
   rewrite(fout);
   i:=0;
   notin:=true;
   NO :=0;
   N00:=0;
   N10:=3.22e9;
   N20:=2.3e8;
   N30:=0;
   Dt:=0.25/60;
   writeln(fout,i*Dt ,no, n00, n10, n20, n30);
   readln(fin,tempold);
   while not eof(fin) do
   Begin
      i:=i+1;
      readln(fin,temp);
      AvTemp:=(Temp+Tempold)/2;
      KKd2:=Kd2(AvTemp);
      KKa:=Ka(AvTemp);
      KKd1:=Kd1(AvTemp);
      KK:=KKa/(KKd1+KKa-KKd2);
        if (NO < 1e-10) then NO:=0;
             NN:=NO*exp(-KKd2*Dt);
```

```
      if (N00 < 1e-10) then N00:=0;
          N0:=N00+N10*KKd1*(1-exp(-(KKd1+KKa)*Dt))/(KKd1+KKa);
      if (N10 <= 1e-10) then N10:=0.0;
          N1:=N10*exp(-(KKA+KKd1)*Dt);
      if (N20 <= 1e-10) then N20:=0.0;
          N2:=(N20+N10*KK)*exp(-KKd2*Dt)
              -N10*KK*exp(-(KKd1+KKa)*Dt);
      if (N30 <= 1e-10) then N30:=0.0 ;
          N3:=N30+N20*(1-Exp(-KKd2*Dt))+N10*KK*(1-exp(-KKd2*Dt))
              -N10*KK*KKd2*(1-exp(-(KKd1+KKa)*dt))/(KKd1+KKa);
    writeln(fout,i*dt  ,NN, N0, N1, N2, N3);
    tempold:=temp;
    if notin then
     begin
      if N2<N20 then
      begin
        NN:=N2;
        notin:=false;
      end;
     end;
    NO:=NN;
    N00:=N0;
    N10:=N1;
    N20:=N2;
    N30:=N3;
  End;
 close(fin);
 close(fout);
end.
```

APPENDIX IV
COMPOSITIONS

A. Composition of 1.5% Nutrient agar

Pancreatic Digest of Gelatin	5.0 g
Beef Extract	3.0 g
Sodium Chloride	8.0 g
Agar	15.0 g

Suspend 31 g in one Liter purified water
pH 7.3±0.2

B. Composition of 0.1M phosphate buffered saline (PBS)

Na_2HPO_4	14.2 g
NaH_2PO_4	6.0 g
NaCl	11.7 g
Distilled water	1.3 L

Adjust pH ≈ 7.3

C. Composition of minimal media

Solution A (pH=7.0)

Potassium dihydrogen phosphate	3.0 g
Disodium hydrogen phosphate	6.0 g
Ammonium chloride	2.0 g
Sodium chloride	5.0 g
Distilled water	800 ml

Solution B (pH=7.0)

Glucose	8.0 g
$MgSO_4.7H_2O$	0.1 g
Agar	15.0 g
Distilled water	200 ml

Autoclave solutions A and B separately.

Source: Microbiology a laboratory manual
Authors: James G. Cappuccino, Natalie Sherman
Addison-Wesley Publishing Company-1983, p455

APPENDIX V

A: Temperature History For Model Validation (Fig. 4.32). Scan Rate ¼th Second. Read temperature by line, starting at time t=0

100.1 100.1 100.1 100.2 100.1 100.2 100.1 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2
100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.2 100.3 100.3 100.3 100.3 100.2 100.2 100.2 100.2
100.2 100.3 100.3 100.3 100.3 100.3 100.3 100.3 100.3 100.3 100.3 100.3 100.4 100.4 100.4 100.5 100.5 100.5
100.6 100.6 100.6 100.6 100.6 100.6 100.7 100.7 100.7 100.8 100.8 100.9 100.9 100.9 100.9 100.9 100.9 100.9
101.0 100.9 100.8 100.8 100.9 100.9 100.9 100.9 100.9 101.0 101.0 101.0 101.0 100.9 100.8 100.8 100.8 100.8
100.8 100.8 100.9 100.8 100.8 101.0 101.0 101.0 101.0 101.0 101.1 101.1 101.0 101.0 101.0 101.0 101.0 101.0
101.0 101.0 101.0 101.0 101.0 101.0 101.0 101.3 101.4 101.4 101.3 101.4 101.5 101.4 101.5 101.5 101.4 101.4
101.5 101.5 101.6 101.6 101.6 101.6 101.6 101.7 101.7 101.7 101.7 101.7 101.8 101.8 101.8 101.8 101.8 101.7
101.7 101.6 101.6 101.7 101.7 101.7 101.7 101.7 101.8 101.9 101.9 101.9 101.9 101.9 102.0 102.0 102.0 102.1
102.2 102.2 102.2 102.2 102.2 102.1 102.1 102.1 102.2 102.1 102.2 102.1 102.1 102.1 102.2 102.2 102.2 102.2
102.2 102.2 102.2 102.2 102.2 102.2 102.3 102.3 102.3 102.3 102.3 102.3 102.3 102.4 102.4 102.4 102.4 102.4
102.5 102.5 102.6 102.6 102.6 102.7 102.7 102.7 102.7 102.7 102.7 102.7 102.8 102.8 102.9 102.9 102.9 102.9
102.9 102.9 102.9 102.9 102.9 103.0 103.1 103.1 103.2 103.2 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3
103.2 103.2 103.1 103.1 103.1 103.1 103.1 103.2 103.2 103.2 103.2 103.2 103.3 103.2 103.3 103.3 103.3 103.3
103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.3 103.4 103.3
103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.4 103.5 103.4 103.5 103.5
103.5 103.5 103.5 103.5 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.6
103.6 103.6 103.6 103.6 103.6 103.6 103.6 103.7 103.7 103.7 103.7 103.7 103.7 103.7 103.8 103.7 103.8 103.8
103.8 103.8 103.8 103.8 103.8 103.8 103.9 103.8 103.9 103.9 103.9 103.9 103.9 103.9 103.9 103.9 103.9 103.9
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103.8 103.9 103.9 103.9 103.8 103.8 103.8 103.8 103.8 103.8 103.8 103.8 103.8 103.9 103.9 103.9 104.0 104.0
104.0 104.0 104.0 104.0 104.0 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.1 104.2
104.2 104.2 104.2 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.3 104.2
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104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5 104.5
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104.5 104.5 104.4 104.4 104.4 104.5 104.5 104.5 104.5 104.5 104.5 104.4 104.6 104.6 104.6 104.6 104.6 104.6
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104.6 104.6 104.6 104.6 104.6 104.6 104.5 104.5 104.5 104.6 104.6 104.6 104.6 104.6 104.6 104.6 104.6 104.6
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108.5	108.5	108.4	108.4	108.4	108.4	108.5	108.6	108.6	108.7	108.8	108.7	108.7	108.7	108.7	108.8	108.7	108.8	108.8
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B. Temperature History for Model Validation (Fig. 4.35). Scan Rate ¼th Second. Read temperature by line, starting at time t=0

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109.3	109.3	109.3	109.3	109.3	109.4	109.4	109.4	109.4	109.5	109.5	109.5	109.5	109.4	109.4	109.4	109.5	109.4
109.5	109.5	109.4	109.4	109.4	109.4	109.4	109.5	109.5	109.4	109.5	109.5	109.5	109.4	109.4	109.4	109.5	109.5
109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.5	109.6	109.6	109.6	109.6	109.5	109.5
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107.9	107.9	107.9	107.9	107.9	107.9	108.0	107.9	108.0	108.0	108.0	108.0	108.0	108.0	108.1	108.1	108.2	108.2	108.2
108.2	108.2	108.1	108.2	108.2	108.3	108.3	108.3	108.3	108.3	108.3	108.3	108.4	108.5	108.6	108.5	108.6	108.8	
108.8	108.8	108.8	108.9	108.8	108.7	108.6	108.7	108.7	108.8	108.9	108.9	108.9	108.9	108.9	108.8	108.9	108.8	108.8
108.7	108.9	109.0	109.1	109.1	109.2	109.2	109.1	109.1	109.1	109.1	109.1	109.0	109.1	109.1	109.1	109.1	109.1	
109.1	109.0	109.0	109.1	109.0	109.0	109.0	109.0	108.9	108.8	108.8	108.7	108.7	108.7	108.7	108.7	108.7	108.7	
108.8	108.9	109.0	109.1	109.1	109.3	109.2	109.2	109.1	109.1	109.3	109.4	109.4	109.4	109.5	109.5	109.5	109.4	
109.3	109.3	109.2	109.2	109.2	109.2	109.1	109.1	109.1	109.0	109.0	109.0	109.0	108.9	108.9	108.9	109.1	109.1	
109.1	109.0	109.1	109.0	109.1	109.1	109.3	109.4	109.5	109.5	109.4	109.3	109.3	109.2	109.2	109.2	109.2	109.1	109.2
109.3	109.3	109.3	109.3	109.3	109.3	109.4	109.5	109.6	109.5	109.5	109.6	109.6	109.5	109.5	109.4	109.4	109.3	
109.3	109.4	109.3	109.4	109.5	109.6	109.6	109.6	109.6	109.6	109.6	109.6	109.6	109.5	109.6	109.7	109.8	109.9	
109.9	109.9	109.9	109.9	109.9	110.0	110.0	110.0	109.9	109.8	109.8	109.6	109.8	109.8	109.8	109.8	109.8	109.7	
109.7	109.6	109.7	109.8	109.9	110.0	110.0	110.1	110.1	110.0	109.9	109.9	110.1	110.1	110.2	110.1	110.2	110.1	
110.1	110.2	110.2	110.1	110.2	110.2	110.2	110.1	110.2	110.3	110.3	110.4	110.4	110.4	110.3	110.4	110.5	110.5	
110.5	110.5	110.4	110.4	110.3	110.2	110.1	110.0	110.1	110.1	110.1	110.1	110.2	110.3	110.4	110.4	110.5	110.5	
110.4	110.4	110.4	110.5	110.5	110.6	110.6	110.7	110.7	110.7	110.7	110.7	110.7	110.7	110.8	110.8	110.8	110.8	
110.8	110.8	110.8	110.8	110.9	110.9	110.9	111.0	111.1	111.2	111.0	110.9	110.9	110.9	111.0	111.0	111.1	111.1	
111.1	111.0	111.1	111.1	111.1	111.0	111.0	111.0	111.1	111.1	111.0	111.0	111.0	111.0	110.9	110.9	110.9	110.8	
110.8	110.8	110.8	110.8	110.8	111.0	111.1	111.2	111.1	111.2	111.3	111.2	111.2	111.2	111.1	111.1	111.2	111.1	
111.2	111.2	111.2	111.2	111.2	111.2	111.1	111.2	111.1	111.1	111.2	111.2	111.2	111.2	111.3	111.2	111.2	111.2	
111.2	111.2	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.4	111.5	111.7	111.8	111.9	111.9
111.9	111.9	111.9	111.9	111.9	112.0	112.0	112.0	112.0	112.1	112.1	112.2	112.2	112.1	111.9	111.9	111.8	111.8	
111.8	111.8	111.8	111.8	111.8	111.8	111.7	111.8	111.8	111.8	111.8	111.8	111.8	111.8	111.8	111.8	111.9	111.8	
111.9	112.0	112.0	111.9	111.9	111.9	111.9	112.0	112.1	112.1	112.1	112.2	112.2	112.2	112.2	112.2	112.2	112.2	
112.2	112.3	112.3	112.3	112.3	112.5	112.7	112.8	112.8	112.9	113.1	113.1	113.1	113.1	113.2	113.2	113.2	113.2	
113.2	113.2	113.2	113.2	113.1	113.0	113.0	113.0	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	113.1	
113.0	113.1	113.1	113.1	113.1	113.0	112.9	113.0	113.0	113.0	113.0	112.9	112.9	112.9	112.9	112.9	113.0	113.0	
113.1	113.1	113.1	113.0	113.0	113.1	113.1	113.2	113.2	113.2	113.2	113.1	113.2	113.1	113.1	113.1	113.1	113.2	
113.2	113.2	113.2	113.2	113.2	113.2	113.2	113.2	113.2	113.2	113.2	113.4	113.5	113.5	113.7	113.7	113.8	113.8	
113.7	113.6	113.6	113.5	113.5	113.5	113.5	113.4	113.4	113.4	113.4	113.4	113.4	113.3	113.3	113.4	113.6	113.6	113.6
113.7	113.9	114.0	114.0	114.0	114.2	114.1	114.0	114.0	113.9	113.9	113.9	113.9	114.0	114.0	114.0	114.0	114.0	
114.0	114.2	114.2	114.1	114.1	114.1	114.1	114.0	114.0	114.0	114.0	114.1	114.1	114.0	113.9	113.9	113.9	114.0	
114.1	114.2	114.2	114.3	114.4	114.4	114.5	114.5	114.6	114.6	114.5	114.5	114.6	114.6	114.7	114.6	114.5		
114.6	114.6	114.8	114.7	114.6	114.6	114.5	114.5	114.4	114.4	114.4	114.5	114.5	114.5	114.5	114.5	114.6	114.8	
114.8	114.9	114.8	114.8	114.7	114.7	114.8	114.9	115.0	115.0	114.9	114.8	115.0	115.1	115.1	115.0	115.0	115.1	
115.1	115.0	114.9	115.0	114.9	115.0	115.0	115.0	115.2	115.2	115.2	115.2	115.2	115.1	115.1	115.0	115.0	115.1	
115.3	115.3	115.2	115.1	115.2	115.2	115.2	115.2	115.2	115.3	115.4	115.5	115.5	115.5	115.4	115.4	115.4	115.4	
115.3	115.2	115.2	115.3	115.3	115.3	115.3	115.3	115.3	115.3	115.4	115.4	115.4	115.5	115.5	115.5	115.5	115.5	
115.5	115.5	115.5	115.7	115.9	115.9	116.0	115.9	115.9	115.9	115.9	115.9	115.9	115.9	115.9	115.9	115.9	115.9	
116.0	115.9	115.9	116.0	116.0	116.0	116.0	116.1	116.2	116.1	116.1	116.1	116.1	116.0	116.0	116.1	116.1	116.1	
116.1	116.1	116.1	116.0	116.0	115.9	115.9	115.9	115.9	116.0	116.0	116.1	116.2	116.4	116.4	116.3	116.4		
116.5	116.5	116.6	116.6	116.6	116.6	116.5	116.4	116.3	116.3	116.3	116.2	116.2	116.2	116.2	116.2	116.2	116.2	
116.2	116.2	116.3	116.3	116.4	116.5	116.5	116.5	116.6	116.7	116.6	116.6	116.5	116.5	116.5	116.5	116.6	116.6	
116.6	116.6	116.5	116.6	116.6	116.5	116.6	116.5	116.5	116.5	116.5	116.6	116.6	116.7	116.7	116.7	116.7	116.7	
116.8	116.8	116.8	116.8	116.8	116.9	116.9	117.0	117.0	117.0	116.9	116.9	116.9	117.0	116.9	116.9	116.9	117.0	
117.0	117.0	117.0	117.0	117.0	117.1	117.2	117.2	117.3	117.3	117.3	117.2	117.1	117.1	117.1	117.1	117.0	117.0	
117.1	117.2	117.2	117.2	117.3	117.3	117.3	117.3	117.3	117.3	117.3	117.3	117.3	117.2	117.2	117.3	117.3	117.2	
117.2	117.3	117.3	117.3	117.4	117.6	117.6	117.7	117.6	117.7	117.7	117.7	117.7	117.7	117.7	117.6	117.7	117.6	
117.6	117.5	117.5	117.5	117.6	117.6	117.6	117.6	117.7	117.8	117.8	117.8	117.9	118.1	118.3	118.4	118.5	118.4	
118.3	118.2	118.1	118.1	118.1	118.2	118.2	118.3	118.2	118.2	118.1	118.1	118.0	117.9	117.9	117.9	117.9	117.9	
117.9	117.9	117.9	118.0	117.9	118.1	118.1	118.1	118.0	118.0	118.1	118.1	118.1	118.2	118.3	118.4	118.4	118.5	
118.5	118.6	118.6	118.6	118.6	118.7	118.7	118.5	118.5	118.7	118.6	118.5	118.4	118.3	118.3	118.3	118.4	118.4	
118.4	118.5	118.7	118.8	118.8	118.8	118.7	118.6	118.5	118.6	118.6	118.5	118.5	118.6	118.6	118.7	118.7	118.8	
118.9	118.8	118.7	118.6	118.6	118.6	118.6	118.5	118.4	118.4	118.4	118.4	118.4	118.4	118.4	118.4	118.5	118.6	118.6
118.6	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.6	118.7	118.6	118.6	118.6	118.6	118.6	118.6	
118.5	118.4	118.4	118.4	118.4	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.5	118.6	
118.6	118.6	118.6	118.6	118.6	118.6	118.6	118.7	118.7	118.8	118.9	118.9	118.9	119.1	119.1	119.0	119.0	119.0	
119.0	119.1	119.0	119.0	119.0	119.1	119.1	119.0	119.0	119.0	119.0	119.0	119.0	119.0	119.0	119.0	119.1	119.1	
119.1	119.1	119.0	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.2	119.2	119.2	119.1	
119.2	119.3	119.3	119.4	119.4	119.4	119.3	119.4	119.4	119.4	119.3	119.3	119.3	119.4	119.4	119.4	119.4	119.3	
119.4	119.4	119.4	119.5	119.6	119.6	119.5	119.5	119.5	119.5	119.5	119.6	119.7	119.7	119.7	119.7	119.7	119.7	
119.8	119.8	119.8	119.9	119.9	119.9	119.8	119.8	119.8	119.8	119.8	119.8	119.8	119.8	119.8	119.8	119.9	119.9	

119.9	119.9	119.9	119.9	119.8	119.9	120.0	120.1	120.1	120.2	120.3	120.3	120.2	120.2	120.2	120.2	120.3	120.3
120.3	120.4	120.4	120.3	120.3	120.2	120.2	120.2	120.2	120.3	120.2	120.2	120.1	120.1	120.2	120.2	120.2	120.3
120.3	120.3	120.3	120.3	120.3	120.3	120.3	120.4	120.4	120.4	120.5	120.4	120.5	120.4	120.4	120.4	120.4	120.5
120.4	120.4	120.5	120.5	120.6	120.6	120.6	120.5	120.5	120.5	120.5	120.5	120.5	120.5	120.6	120.6	120.6	120.7
120.7	120.7	120.7	120.7	120.7	120.8	120.8	120.9	120.9	121.0	121.0	121.2	121.2	121.2	121.3	121.4	121.3	121.2
121.2	121.1	121.0	120.9	120.8	120.8	120.7	120.7	120.6	120.6	120.6	120.4	120.4	120.4	120.3	120.4	120.4	120.4
120.5	120.5	120.3	120.2	120.1	120.2	120.3	120.3	120.3	120.4	120.4	120.4	120.1	120.1	120.1	120.2	120.1	120.0
120.0	120.1	120.2	120.3	120.3	120.3	120.4	120.4	120.4	120.4	120.4	120.3	120.3	120.3	120.3	120.3	120.3	120.3
120.2	120.2	120.2	120.2	120.2	120.2	120.2	120.2	120.1	120.1	120.1	120.1	120.1	120.0	119.9	119.8	119.8	119.7
119.7	119.7	119.6	119.6	119.5	119.4	119.4	119.4	119.3	119.2	119.1	119.1	119.1	119.1	119.2	119.2	119.1	119.0
119.0	119.2	119.3	119.3	119.2	119.2	119.2	119.2	119.2	118.9	118.8	118.8	118.7	118.9	119.1	119.2	119.2	119.1
119.1	119.1	119.1	119.1	119.1	119.2	119.2	119.3	119.2	119.3	119.3	119.3	119.2	119.1	119.1	119.1	119.2	119.2
119.2	119.0	118.8	118.9	118.9	118.9	118.9	118.9	119.1	118.9	118.6	118.6	118.6	118.8	118.8	118.7	118.8	118.6
118.5	118.5	118.4	118.3	118.4	118.4	118.3	118.4	118.4	118.4	118.3	118.3	118.3	118.2	118.4	118.5	118.6	118.6
118.6	118.5	118.6	118.4	118.2	118.2	118.2	118.2	118.2	118.3	118.3	118.3	118.3	118.4	118.4	118.5	118.6	
118.4	118.4	118.3	118.3	118.1	118.2	118.0	118.0	117.9	117.9	118.1	118.0	117.9	117.8	117.8	117.8	117.8	117.9
117.9	117.9	117.8	117.8	117.9	118.0	118.1	118.1	118.0	118.0	118.1	118.1	118.1	118.0	118.0	118.0	117.9	117.8
117.7	117.6	117.6	117.7	117.8	117.9	117.8	117.9	117.8	117.6	117.6	117.8	117.7	117.8	117.8	117.9	117.9	117.9
117.9	118.0	118.0	118.0	117.9	117.9	117.9	117.6	117.5	117.4	117.3	117.3	117.2	117.2	117.2	117.4	117.5	117.5
117.6	117.6	117.5	117.4	117.4	117.1	117.1	117.3	117.3	117.3	117.3	117.4	117.4	117.5	117.3	117.3	117.3	117.4
117.4	117.4	117.5	117.5	117.5	117.5	117.5	117.4	117.4	117.4	117.5	117.4	117.5	117.5	117.4	117.4	117.4	117.4
117.4	117.3	117.3	117.3	117.2	117.0	116.8	116.9	116.8	117.0	117.0	117.1	117.1	117.2	117.2	117.2	117.2	117.2
117.2	117.1	117.2	117.1	117.0	117.1	117.1	117.1	117.1	117.2	117.3	117.4	117.3	117.2	117.1	117.1	117.1	117.1
117.1	117.1	117.0	117.0	117.0	117.0	117.0	117.0	117.0	116.9	116.9	116.9	117.0	117.1	117.2	117.2	117.3	117.3
117.4	117.3	117.4	117.4	117.4	117.5	117.5	117.5	117.6	117.6	117.6	117.6	117.5	117.6	117.6	117.8	117.8	117.9
117.8	117.7	117.6	117.5	117.5	117.4	117.3	117.2	117.3	117.4	117.5	117.6	117.6	117.5	117.6	117.6	117.6	117.5
117.4	117.2	117.2	117.3	117.4	117.4	117.4	117.5	117.5	117.6	117.6	117.7	117.7	117.7	117.6	117.5	117.5	117.6
117.6	117.7	117.7	117.7	117.8	117.8	117.8	117.8	117.7	117.7	117.6	117.7	117.7	117.7	117.6	117.7	117.7	117.6
117.6	117.6	117.6	117.7	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.7	117.8
117.8	117.8	117.9	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.8	117.9	117.9	117.9	118.0	118.0	118.0	117.9
118.0	118.0	117.9	117.9	117.8	117.8	117.8	117.8	117.7	117.7	117.7	117.8	117.7	117.7	117.7	117.7	117.7	117.8
117.8	117.9	117.8	117.8	117.8	117.6	117.6	117.7	117.7	117.8	117.9	117.9	118.0	118.0	118.1	118.1	118.1	118.1
118.1	118.1	118.1	118.0	118.0	117.9	117.8	117.7	117.7	117.7	117.6	117.7	117.7	117.8	117.8	117.8	117.8	117.9
118.0	118.0	118.0	118.1	118.1	118.1	118.1	118.1	118.1	118.2	118.2	118.2	118.2	118.2	118.2	118.2	118.2	118.3
118.3	118.3	118.3	118.3	118.2	118.2	118.1	118.0	118.1	117.9	117.8	117.8	118.0	118.1	118.0	118.0	118.0	118.1
118.1	118.1	118.2	118.2	118.2	118.3	118.3	118.3	118.4	118.4	118.5	118.5	118.6	118.5	118.4	118.4	118.4	118.4
118.5	118.6	118.5	118.5	118.5	118.5	118.5	118.5	118.4	118.4	118.5	118.6	118.6	118.7	118.7	118.6	118.6	118.6
118.6	118.6	118.6	118.5	118.4	118.4	118.4	118.4	118.4	118.3	118.3	118.4	118.5	118.5	118.6	118.6	118.7	
118.8	118.8	118.6	118.6	118.7	118.9	119.0	119.0	119.1	119.1	119.2	119.1	119.2	119.2	119.3	119.1	119.1	119.0
118.9	119.0	119.1	119.1	119.2	119.2	119.1	119.1	119.2	119.2	119.3	119.3	119.2	119.0	118.9	118.7	118.6	118.6
118.6	118.7	118.7	118.7	118.7	118.7	118.8	118.8	118.7	118.7	118.8	118.7	118.8	118.8	118.8	118.9	118.9	118.8
118.8	118.8	118.8	118.8	118.8	118.8	118.7	118.7	118.7	118.7	118.7	118.6	118.6	118.6	118.6	118.7	118.7	118.7
118.7	118.7	118.7	118.7	118.7	118.7	118.8	118.8	118.8	118.8	118.8	118.8	118.8	118.8	118.7	118.7	118.7	118.7
118.6	118.5	118.6	118.6	118.6	118.6	118.6	118.6	118.7	118.6	118.7	118.7	118.8	118.7	118.8	118.8	118.7	118.6
118.6	118.5	118.5	118.5	118.7	118.8	118.8	118.8	118.9	119.0	119.0	119.0	119.0	118.9	118.9	118.9	119.0	119.0
119.1	119.1	119.1	119.1	119.0	118.9	118.8	118.9	119.0	119.1	119.1	119.2	119.3	119.3	119.3	119.3	119.4	119.4
119.4	119.2	119.1	119.1	119.1	118.9	118.9	118.8	118.9	119.0	119.1	119.1	119.2	119.2	119.1	119.2	119.2	119.1
118.9	118.7	118.8	119.0	119.0	119.0	119.1	119.1	119.2	119.3	119.3	119.4	119.6	119.6	119.7	119.8	119.8	119.6
119.7	119.6	119.7	119.7	119.8	119.8	119.9	119.7	119.6	119.6	119.6	119.5	119.2	119.1	119.1	119.1	119.1	119.0
119.0	119.1	119.1	119.1	119.2	119.2	119.2	119.1	119.2	119.3	119.5	119.5	119.4	119.4	119.4	119.4	119.4	119.4
119.3	119.3	119.3	119.3	119.1	119.1	119.1	119.1	119.1	119.0	119.1	119.1	119.2	119.2	119.2	119.3	119.3	119.3
119.3	119.3	119.3	119.3	119.3	119.2	119.2	119.2	119.3	119.3	119.3	119.2	119.2	119.1	119.1	119.2	119.2	119.2
119.3	119.3	119.4	119.4	119.4	119.5	119.5	119.6	119.8	119.8	119.8	119.9	119.8	119.7	119.4	119.3	119.2	119.2
119.3	119.2	119.1	119.1	119.0	119.0	118.9	119.0	119.0	119.1	119.1	119.1	119.1	119.1	119.1	119.2	119.2	119.1
119.0	118.9	118.8	118.9	119.0	119.1	119.1	119.1	118.9	119.0	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1
119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.1	119.0	119.0	119.0	119.0
118.9	118.9	118.9	118.9	118.8	118.7	118.7	118.7	118.7	118.8	118.8	118.8	118.8	118.8	118.8	118.8	118.7	118.5
118.5	118.4	118.5	118.5	118.3	118.1	117.9	118.0	117.8	117.7	117.7	117.7	117.6	117.7	117.5	117.7	117.8	118.0
118.0	117.9	117.9	117.9	118.0	118.0	118.0	118.0	118.1	118.2	118.2	118.0	117.9	117.7	117.7	117.7	117.8	117.8
117.7	117.7	117.7	117.7	117.8	117.9	117.8	117.9	117.9	118.0	118.1	118.1	118.1	118.2	118.1	118.0	117.9	117.9
117.7	117.6	117.7	117.8	117.9	117.9	118.0	118.0	117.9	117.8	117.7	117.8	117.8	117.8	117.9	117.8	117.9	117.9
118.0	118.0	117.9	117.8	117.7	117.8	117.8	117.8	117.8	117.8	117.8	117.9	117.9	118.0	118.0	118.0	118.0	118.0

118.0 118.0 118.0 118.0 118.0 118.1 118.1 118.1 118.1 118.1 118.1 118.2 118.2 118.2 118.2 118.2 118.2 118.3
118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.3 118.2 118.2 118.3 118.3 118.3 118.3
118.3 118.3 118.3 118.3 118.3 118.3 118.4 118.4 118.4 118.4 118.4 118.4 118.4 118.5 118.6 118.7 118.7 118.7
118.6 118.7 118.7 118.7 118.7 118.8 118.8 118.8 118.8 118.8 118.8 118.8 118.8 118.8 118.8 118.8 118.9 118.9
118.9 118.8 118.8 118.8 119.0 119.0 119.0 119.1 119.1 119.1 119.1 119.1 119.1 119.0 119.0 119.0 119.1 119.1
119.1 119.1 119.1 119.2 119.3 119.3 119.3 119.2 119.2 119.2 119.2 119.2 119.2 119.2 119.3 119.4 119.4 119.4
119.5 119.5 119.5 119.6 119.7 119.8 119.8 119.7 119.7 119.8 119.7 119.7 119.7 119.8 119.7 119.7 119.6 119.6
119.6 119.6 119.6 119.6 119.7 119.8 119.8 119.8 119.9 119.9 120.0 120.1 120.2 120.1 120.1 120.0 119.9 119.9
119.9 120.0 120.1 120.2 120.1 120.1 120.1 120.1 120.1 120.1 120.0 120.0 120.0 120.0 120.0 120.0 120.0 120.0
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120.3 120.3 120.4 120.4 120.5 120.5 120.5 120.4 120.5 120.5 120.6 120.7 120.8 120.8 120.7 120.7 120.7 120.7
120.6 120.6 120.6 120.6 120.6 120.6 120.6 120.6 120.6 120.6 120.7 120.7 120.7 120.7 120.7 120.7 120.7 120.8
120.8 120.8 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9 120.9
120.8 120.9 121.0 121.0 121.1 121.1 121.1 121.1 121.1 121.2 121.2 121.2

C. Ultra High Temperature Histories (Capillary Experiments). Scan rate ⅛th Second. Read temperatures by line, starting at time t=0.

C1: Temperature History (Fig. 4.41)

26.5 26.5 26.5 26.5 26.5 27.1 27.1 27.1 27.1 27.1 27.1 27.1 27.0 27.1 27.1 27.0 27.0 27.0
27.0 27.0 27.0 27.1 27.0 27.0 27.0 27.1 27.2 27.2 27.2 27.3 27.3 27.3 27.3 27.4 27.4 27.4 27.4 27.4
27.4 27.4 27.4 27.5 27.6 28.0 28.7 37.4 51.2 63.5 74.7 84.3 92.3 98.6 103.7 107.9 111.3 114.0
116.1 117.8 119.2 120.2 121.2 122.0 122.7 123.3 123.7 124.1 124.4 124.7 125.0 125.2 125.4 125.6 125.7 125.8
126.0 126.1 126.2 126.2 126.3 126.3 126.4 126.4 126.4 126.4 126.5 126.5 126.5 126.6 126.6 126.6 126.7 126.7
126.8 126.8 126.9 126.9 126.9 126.9 126.9 126.9 127.0 127.0 127.0 127.0 127.0 127.0 127.0 126.9 126.9 126.9
126.9 126.9 126.8 126.3 125.8 125.0 124.2 123.3 122.4 120.9 119.0 117.5 115.6 114.1 112.8 111.9 110.9 110.4
109.5 109.2 108.5 107.8 107.0 106.5 105.8 105.1 104.4 103.7 103.6 103.3 102.9 102.4 101.5 100.8 100.0 99.4
98.8 98.0 97.5 96.5 96.0 95.5 94.8 94.4 94.2 93.9 93.5 93.1 92.7 92.3 92.2 91.8 91.4 91.0 90.7 90.4
90.0 89.6 89.2 88.7 88.2 87.8 87.3 86.8 86.4 86.0 85.7 85.4 85.1 84.9 84.7 84.5 84.3 84.0 83.7 83.4
83.1 82.6 82.1 81.6 81.1 80.7 80.3 79.9 79.6 79.3 78.9 78.7 78.4 78.2 78.0 77.8 77.7 77.6 77.5 77.2
76.9 76.7 76.5 76.2 75.9 75.5 75.2 74.8 74.5 74.3 74.1 74.0 73.8 73.7 73.5 73.3 73.1 73.0 72.8 72.8
72.6 72.5 72.4 72.4 77.5 84.2 91.2 97.3 102.8 107.6 111.5 114.7 117.1 119.1 120.7 121.9 122.9 123.8 124.5
125.0 124.7 123.4 122.3 121.2 119.9 118.7 117.5 116.4 115.5 114.7 114.2 113.7 113.2 112.5 111.9 111.1 110.5
109.8 109.1 108.4 108.2 107.8 107.4 106.7 106.3 104.3 101.7 99.3 97.0 95.2 93.5 91.8 90.6 89.3 88.6 88.0
87.5 87.0 86.3 85.7 85.1 84.7 84.3 84.0 83.8 83.5 83.3 83.2 83.2 83.1 83.1 83.0 82.9 82.8 82.7 82.6
82.3 82.1 82.0 81.8 81.7 81.5 81.4 81.3 81.1 80.8 80.7 80.6 80.5 80.4 80.4 80.3 80.2 79.9 79.7 79.3
78.9 78.5 78.2 77.8 77.4 77.0 76.6 76.2 75.7 75.2 74.9 74.7 74.4 74.1 73.8 73.5 73.0 72.4 70.8 62.4
54.0 47.2 42.2 38.4 35.2 32.9 31.0 29.4 28.2 27.2 26.4 25.9 25.5 25.0 24.7 24.4 24.1 23.9 23.7 23.5
23.3 23.2 23.0 22.9 22.8 22.7 22.6 22.6 22.5 22.4 22.4 22.3 22.3 22.2 22.2 22.2 22.1 22.1 22.1
22.0 21.9 21.9 21.8 21.8 21.8 21.7 21.7 21.6 21.6 21.6 21.6 21.6 21.6 21.6 21.5 21.5 21.5 21.4 21.4
21.3 21.3 21.3 21.3 21.3 21.2 21.2 21.2

C2. Temperature History (Fig. 4.42)

21.2 21.2 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3 21.3
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88.7 94.3 98.6 102.2 105.3 108.0 110.2 112.4 114.2 115.7 117.1 118.2 119.1 120.0 120.4 120.8 121.3 121.7 122.0
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122.5 122.5 122.5 122.4 121.9 120.8 120.0 119.0 117.3 115.9 114.6 113.5 112.8 112.2 111.6 111.0 110.2 109.7
108.9 108.1 107.2 106.4 105.6 104.5 103.3 102.4 101.6 101.0 100.4 100.0 99.7 99.3 98.9 98.5 98.1 97.7 97.4
97.1 96.9 96.6 96.3 96.1 95.9 95.7 95.3 94.8 94.3 93.7 93.1 92.5 91.8 91.2 90.6 90.1 89.5 88.9
88.5 87.8 87.0 86.2 85.7 85.1 84.8 84.5 76.1 60.8 49.4 42.5 37.3 33.3 30.5 28.5 27.0 25.8 25.0 24.3
23.8 23.4 23.2 22.9 22.7 22.6 22.4 22.2 22.2 22.1 22.0 22.0 22.0 22.0 22.0 22.0 22.1 22.1
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C3. Temperature History (Fig. 4.43)

21.6 21.6 21.6 21.6 21.6 27.7 27.8 27.8 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27.7 27.7
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28.0 27.9 28.0 28.0 28.0 28.0 28.1 28.1 28.2 28.3 28.5 31.1 39.9 51.0 61.2 70.5 78.5 85.7 91.7 96.7
100.9 104.7 108.1 111.0 113.6 115.9 117.8 119.5 120.8 122.0 122.9 123.7 124.4 124.9 125.4 125.8 126.1 126.4
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117.8 116.7 115.7 114.7 113.7 112.8 111.9 111.4 110.8 110.1 108.9 107.5 106.1 104.9 103.5 102.4 101.3 100.4
99.5 98.8 98.2 97.7 97.1 96.4 95.8 95.2 86.2 72.7 60.8 51.6 45.1 40.8 37.7 35.5 33.9 32.7 31.8 31.1
30.5 30.1 29.8 29.6 29.4 29.2 29.0 28.9 28.8 28.7 28.6 28.5 28.5 28.4 28.3 28.3 28.3 28.3 28.3
28.2 28.2 28.2 28.2 28.1 28.1 28.1 28.1 28.1 28.1 28.1 28.1 28.1 28.1 28.0 28.1 28.0 28.0 28.0
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C4. Temperature History (Fig. 4.44)

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25.8 25.8 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 26.0 26.0 26.0 26.0
26.1 26.1 26.1 26.1 26.2 26.2 26.2 26.3 26.3 26.3 26.3 26.4 26.4 26.4 26.4 26.4 26.4 26.4 26.5
26.5 26.5 26.5 26.5 26.5 26.6 26.6 26.6 26.7 26.6 26.7 26.7 26.7 26.7 26.7 26.8 26.8
26.8 26.8 26.8 26.8 26.8 26.8 26.8 26.8 26.9 26.9 26.9 26.9 26.9 26.9 26.9 26.9 26.9 27.0 27.0
27.0 27.0 27.1 27.1 27.1 27.1 27.1 27.1 27.2 27.2 27.2 27.2 27.3 27.5 30.5 41.4 53.7 66.1 77.2

86.2 93.8 100.1 105.3 109.5 113.0 115.9 118.3 120.4 122.0 123.5 124.7 125.7 126.6 127.3 127.9 128.5 128.9 129.2
129.5 129.8 130.0 130.3 130.5 130.6 130.7 130.9 130.9 131.0 131.1 131.1 131.1 131.2 131.2 131.2 131.3 131.3
131.3 131.3 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.5 131.5 131.5 131.4 131.4 131.4 131.5
131.4 131.4 131.4 131.4 131.5 131.5 131.5 131.5 131.5 131.5 131.6 131.6 131.6 131.6 131.6 131.6 131.6 131.7
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132.0 131.9 131.7 131.7 131.7 131.7 131.7 131.7 131.7 131.7 131.7 131.7 131.5 131.0 129.6 127.0 125.4 123.6
121.8 84.9 59.6 49.3 43.9 40.6 38.4 36.5 34.9 34.1 33.7 33.3 32.7 32.1 31.6 31.0 30.5 30.0 29.6
29.4 29.2 29.1 29.0 28.9 28.8 28.8 28.7 28.7 28.6 28.6 28.6 28.5 28.5 28.4 28.4 28.4 28.4 28.4 28.4
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27.8 27.9 27.9 27.9 27.9 27.9 27.9 27.9 27.9 27.9 27.9

C5. Temperature History (Fig. 4.45)

27.9 27.9 27.9 27.9 27.9 27.9 27.9 27.8 27.9 27.9 27.9 27.9 28.4 28.4 28.4 28.4 28.4 28.4 28.3
28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.3 28.4 28.4 28.5 28.7 28.8
28.8 31.4 39.8 50.5 61.0 70.6 79.3 87.1 93.8 99.3 104.0 108.1 111.4 114.2 116.5 118.6 120.3 121.7 122.9
123.9 124.7 125.4 126.1 126.6 127.0 127.4 127.7 128.0 128.2 128.4 128.5 128.7 128.8 128.9 129.0 129.1 129.1
129.2 129.2 129.2 129.3 129.3 129.3 129.3 129.3 129.3 129.4 129.4 129.4 129.4 129.4 129.4 129.4 129.3 129.3
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84.1 74.3 66.0 59.6 54.6 50.4 47.1 44.3 42.1 40.2 38.6 37.4 36.4 35.6 34.8 34.2 33.6 33.1 32.7
32.3 32.0 31.7 31.5 31.3 31.1 30.9 30.8 30.7 30.6 30.6 30.5 30.4 30.4 30.4 30.4 30.4 30.4 30.3
30.3 30.3 30.2 30.2 30.2 30.2 30.2 30.2 30.2 30.2 30.1 30.1 30.1 30.0 30.0 29.9 29.9 29.8
29.8 29.7 29.6 29.6 29.5 29.5 29.4 29.3 29.3 29.2 29.2 29.2 29.1 29.1 29.0 29.0 28.9 28.9 28.8
28.8 28.7 28.8 28.7 28.7 28.6 28.6 28.5 28.5 28.4 28.5 28.4 28.4 28.3 28.3 28.3 28.3 28.2
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C6. Temperature History (Fig. 4.46)

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126.4 126.4 126.4 126.4 126.4 126.4 126.4 126.4 126.4 126.4 126.5 126.4 126.5 126.5 126.4 126.5 126.5 126.5
126.5 126.5 126.5 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.6 126.7 126.7 126.7
126.7 126.7 126.7 126.7 126.7 126.7 126.7 126.7 126.7 126.7 126.7 126.8 126.8 126.8 126.8 126.8 126.8 126.8
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127.3 127.3 127.3 127.3 127.3 127.3 127.3 127.3 127.3 127.3 127.3 127.1 126.3 125.3 123.9 122.6 114.2 98.3
83.3 71.3 60.7 52.3 46.0 41.7 38.6 36.2 34.4 32.8 31.6 30.6 29.8 29.1 28.6 28.2 27.9 27.6 27.4 27.1
26.9 26.8 26.7 26.6 26.5 26.4 26.3 26.3 26.2 26.2 26.1 26.1 26.0 26.0 26.0 25.9 25.9 25.8 25.8
25.8 25.8 25.7 25.7 25.7 25.7 25.6 25.6 25.6 25.6 25.6 25.6 25.6 25.6 25.5 25.5 25.5 25.5 25.5 25.5
25.5 25.5 25.5 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3
25.2 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3 25.3
25.3 25.3 25.2 25.1 25.1 25.1 25.0 24.9

C7. Temperature History (Fig. 4.47)

24.9 24.9 30.6 45.9 61.6 74.1 85.4 94.9 102.3 108.4 113.1 117.0 120.0 122.3 124.2 125.7 126.8 127.7 128.5
129.1 129.5 129.8 130.1 130.3 130.4 130.6 130.7 130.8 131.0 131.0 131.1 131.2 131.2 131.2 131.3 131.3
131.4 131.4 131.4 131.4 131.4 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5
131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5
131.5 131.5 131.5 131.5 131.5 131.5 131.4 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5
131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.5 131.4 131.5 131.5 131.5 131.5 131.5
131.5 131.4 131.5 131.5 131.4 131.4 131.5 131.4 131.4 131.4 131.4 131.4 131.5 131.4 131.4 131.4 131.4 131.4
131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4
131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.4 131.3
131.4 131.4 131.4 131.4 131.4 131.4 131.3 131.3 131.4 131.3 131.4 131.3 131.4 131.3 131.4 131.3 131.3 131.3
131.3 131.3 131.4 131.3 131.3 131.4 131.3 131.3 131.3 131.3 131.3 131.3 131.3 131.3 131.3 131.3 131.3 131.3
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130.0 128.8 127.5 125.6 110.2 93.3 79.0 68.3 60.4 54.3 49.1 45.0 41.8 39.4 37.5 36.1 35.0 34.0 33.2
32.3 31.5 30.8 30.3 29.7 29.3 28.9 28.6 28.3 28.1 28.0 27.8 27.6 27.4 27.2 27.1 27.0 26.8 26.7
26.6 26.6 26.4 26.4 26.3 26.3 26.3 26.2 26.2 26.2 26.2 26.1

C8. Temperature History (Fig. 4.48)

26.1 26.2 26.2 26.1 26.2 26.2 26.1 26.1 26.1 26.0 26.0 25.9 25.9 25.9 25.9 25.8 23.6 23.6 23.6
23.6 23.7 23.7 23.7 23.7 23.7 23.7 23.8 23.8 23.8 23.9 23.9 23.9 23.9 23.9 23.9 23.9 23.9 24.0 24.8
34.5 50.2 65.6 79.2 90.9 99.2 106.6 112.6 117.3 120.8 123.7 126.1 128.1 129.6 130.9 131.8 132.7 133.3 133.8
134.3 134.6 134.8 135.0 135.1 135.2 135.3 135.4 135.4 135.5 135.5 135.6 135.6 135.6 135.7 135.7 135.7 135.7
135.7 135.8 135.8 135.8 135.8 135.8 135.8 135.8 135.9 135.9 135.9 135.9 135.9 135.9 135.9 136.0 136.0 136.0
136.0 136.0 136.0 136.0 136.0 136.0 136.0 136.0 136.0 136.0 136.1 136.1 136.0 136.1 136.1 136.1 136.1 136.1
136.1 136.1 136.1 136.1 136.1 136.2 136.1 136.1 136.2 136.1 136.2 136.2 136.2 136.2 136.2 136.2 136.2 136.2
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136.4 136.4 136.5 136.5 136.4 136.1 135.1 133.8 132.4 131.4 127.0 112.5 96.9 83.9 72.7 63.8 56.4 50.5 46.1
42.8 40.0 37.7 35.8 34.1 32.9 31.9 31.0 30.3 29.7 29.2 28.7 28.4 28.1 27.8 27.6 27.5 27.3 27.1
27.0 26.9 26.9 26.9 26.9 27.0 27.0 27.1 27.1 27.2 27.3 27.3 27.4

C9. Temperature History (Fig. 4.49)

27.4 27.4 27.5 27.5 27.5 27.5 27.5 27.5 27.4 27.4 27.3 27.2 27.1 27.0 26.9 26.8 26.7 26.6 26.5
26.4 26.3 26.3 26.2 26.1 26.1 26.0 26.0 26.0 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.9
25.9 25.9 25.9 25.9 25.9 25.9 25.9 25.8 25.8 25.9 25.8 25.8 25.8 25.8 25.8 27.0 27.0 27.0 27.0
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27.1 27.5 27.8 36.8 55.9 78.6 95.3 104.9 112.5 118.0 122.3 125.6 128.4 130.5 132.2 133.5 134.6 135.4 136.1
136.7 137.1 137.4 137.7 137.9 138.0 138.2 138.3 138.3 138.3 138.4 138.4 138.5 138.5 138.5 138.5 138.5
138.5 138.5 138.5 138.5 138.4 138.4 138.4 138.5 138.5 138.4 138.4 138.4 138.4 138.4 138.4 138.4 138.4
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138.2 138.2 138.2 138.1 138.0 137.3 136.0 134.4 133.3 124.5 104.1 85.7 71.3 60.7 52.7 46.6 41.5 37.5 34.7
32.7 31.1 30.0 29.1 28.5 28.0 27.6 27.2 27.0 26.8 26.6 26.5 26.4 26.3 26.2 26.1 26.0 26.0 25.9
25.9 25.8 25.8 25.7 25.7 25.7 25.6 25.6 25.6 25.6 25.5 25.6 19.9 19.9 19.9 19.9 19.9 20.0 19.9 19.9
19.9 19.9 19.9 19.9 19.9 19.9 19.9

C10. Temperature History (Fig. 4.50)

19.9 20.2 29.8 48.2 69.0 84.9 95.7 103.7 109.7 114.3 118.0 120.9 123.2 125.0 126.5 127.6 128.5 129.2 129.8
130.3 130.6 130.9 131.1 131.3 131.5 131.6 131.7 131.7 131.8 131.8 131.8 131.9 131.9 132.0 132.0 132.0 132.0
132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0 132.0
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132.1 132.1 132.1 132.1 132.1 132.1 132.1 132.1 132.2 132.1 132.1 132.1 132.1 132.2 132.2 132.2 132.2
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132.4 132.4 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5 132.5
132.5 132.5 132.5 132.5 132.5 132.6 132.5 132.5 132.5 132.5 132.5 132.5 132.6 132.6 132.6 132.5 132.5 132.4
131.6 130.9 130.1 129.0 126.8 111.4 92.3 76.8 66.1 57.8 51.3 46.1 42.4 39.6 37.5 35.8 34.6 33.5 32.7
32.0 31.5 31.1 30.7 30.3 29.9 29.5 29.1 28.8 28.3 27.9 27.5 27.2 27.1 27.0 26.9 26.9 26.8 26.7
26.7 26.7 26.6 26.5 26.5 26.4 26.4 26.3 26.3 26.3 26.3 26.2 26.2 26.2 26.2 26.2 26.2 26.1 26.1 26.1
26.1 26.1 26.1 26.1 26.1 26.0 26.1 26.1 26.1 26.0 26.1 26.1 26.0 26.1 26.1 26.0 26.0 26.0 26.0
26.0 26.0 26.0 26.0 26.0 26.0 26.0 26.0

C11. Temperature History (Fig. 4.51)

23.0 23.0 23.0 23.0 23.1 23.1 23.1 23.1 23.2 23.2 23.2 23.3 23.3 23.3 23.3 23.3 23.3 23.3 23.4
23.4 23.4 23.4 23.4 23.5 23.5 23.5 23.6 23.8 23.9 24.4 36.3 63.4 81.4 96.4 109.5 117.2 122.2 125.9
129.5 133.1 135.8 137.8 139.3 140.6 141.6 142.4 143.1 143.6 144.1 144.4 144.7 145.0 145.1 145.2 145.4 145.4
145.4 145.5 145.5 145.2 144.2 142.6 140.8 137.7 120.5 99.5 83.0 70.7 61.4 54.2 48.5 44.0 40.3 37.7 35.8
34.3 33.1 32.0 31.3 30.6 30.1 29.7 29.3 29.0 28.6 28.3 27.9 27.7 27.4 27.2 27.0 26.8 26.7 26.6
26.6 26.6 26.7 26.8 26.8 26.8 26.8 26.8 26.7 26.7 26.6 26.6 26.6 26.5 26.5 26.5 26.5 26.5 26.5
26.4 26.4 26.4 26.4 26.4 26.4 26.3 26.3 26.3 26.3 26.2 26.2 26.2 26.2 26.1 26.1 26.1 26.1 26.0
26.0 26.0 26.0 25.9 25.9 25.9 25.9 25.9 25.9 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8
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25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8
25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8 25.8

D. Ultra High Temperature Histories (Reactor Experiment). Scan rate ½ Second. Read temperature by line starting at time t=0

D1: Temperature History (Fig. 4.52)

89.3 89.3 89.4 89.4 89.4 89.4 89.5 116.9 120.3 121.2 121.5 121.7 121.8 121.9 121.9 121.9 121.9 121.9
121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9
121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9
121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.9 121.8 121.8 121.9 121.8 121.8
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121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4
121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4 121.4
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94.3 93.5 92.7 91.9 91.2 90.6 89.9 89.3 88.7 88.2 87.7 87.3 86.8 86.4 86.0 85.7 85.4 85.1 84.9
84.6 84.4 84.1 83.9 83.7 83.5 83.3 83.2

D2. Temperature History (Fig. 4.54)

95.8	95.7	95.7	95.8	95.7	95.7	95.7	95.7	95.8	119.0	122.0	122.8	123.1	123.1	123.2	123.2	123.2	123.2	123.2
123.2	123.2	123.2	123.2	123.2	123.2	123.2	123.2	123.2	123.3	123.2	123.2	123.2	123.2	123.2	123.2	123.2	123.2	123.2
123.2	123.1	123.2	123.2	123.2	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1	123.1
123.1	123.1	123.1	123.1	123.1	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0
123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	123.0	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9
122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.9	122.8	122.9
122.8	122.8	122.8	122.9	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8
122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.8	122.7	122.7	122.8	122.8	122.7	122.7
122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.7
122.7	122.7	122.7	122.7	122.7	122.7	122.6	122.7	122.7	122.6	122.6	122.6	122.7	122.6	122.6	122.6	122.6	122.6	122.6
122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6	122.6
122.6	122.5	122.5	122.6	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5
122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5	122.5
122.5	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4
122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.4	122.3	122.3
122.3	122.4	122.4	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3
122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3	122.3
122.2	122.2	122.3	122.3	122.2	122.3	122.3	122.3	122.2	122.2	122.2	122.2	122.3	122.3	122.2	122.3	122.2	122.2	122.2
122.2	122.2	122.2	122.2	122.2	122.2	122.2	122.2	122.2	122.1	122.2	122.2	122.2	122.2	122.2	122.2	122.2	122.2	122.1
122.1	122.1	122.2	122.2	122.2	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1
122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1
122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.1	122.0	122.0	122.0	122.1	122.0	122.1	122.0	122.0
122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0
122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0	122.0
121.9	121.9	121.9	121.9	121.9	122.0	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9
121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.9	121.8	121.9	121.8
121.9	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.9	121.8	121.8	121.8	121.8
121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8	121.8
121.8	121.8	121.8	121.7	121.7	121.8	121.8	121.8	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7
121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7
121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.7	121.6	121.7	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6
121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6
121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6	121.6
121.6	121.6	121.6	121.6	121.6	121.5	121.5	121.6	121.5	121.5	121.6	121.5	121.5	121.6	121.5	121.5	121.6	121.5	121.5
121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5
121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.5	121.4	121.4	121.4	121.5	121.4	121.5
121.4	121.4	121.4	121.4	121.5	121.5	121.4	121.5	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4
121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4	121.4
121.4	121.4	121.4	121.3	121.4	121.4	121.3	121.4	121.4	121.3	121.4	121.4	121.3	121.3	121.3	121.3	121.3	121.3	121.3
121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3
121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3
121.3	121.3	121.3	121.3	121.2	121.3	121.3	121.3	121.3	121.3	121.2	121.2	121.2	121.2	121.2	121.3	121.3	121.2	121.2
121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2
121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.1	121.2	121.2	121.2	121.1	121.2
121.2	121.2	121.1	121.2	121.2	121.2	121.2	121.1	121.2	121.1	121.2	121.1	121.2	121.2	121.2	121.2	121.1	121.1	121.1
121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1
121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1	121.1
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121.0	121.0	121.0	121.0	120.9	121.0	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	121.0	120.9	120.9	120.9
120.9	121.0	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9
120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9	120.9
120.9	120.9	120.9	120.9	120.8	120.9	120.8	120.9	120.9	120.9	120.8	120.9	120.8	120.9	120.8	120.9	120.8	120.8	120.8
120.8	120.8	120.8	120.9	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8
120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8
120.8	120.8	120.8	120.7	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.8	120.7	120.7	120.8	120.8	120.7	120.8	120.8
120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7
120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7	120.7

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D3. Temperature History (Fig. 4.55)

95.6 95.6 95.6 95.6 95.6 95.6 95.6 95.6 95.6 119.9 122.7 123.4 123.6 123.7 123.8 123.8 123.8 123.8 123.8
123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.8 123.9 123.9 123.8 123.9 123.9
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123.8 123.8 123.8 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7 123.7
123.7 123.7 123.7 123.6 123.6 123.6 123.6 123.7 123.6 123.6 123.6 123.6 123.6 123.6 123.6 123.6 123.6 123.6
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D4. Temperature History (Fig. 4.56)

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D5. Temperature History (Fig. 4.57)

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BIOGRAPHICAL SKETCH

Vimal Sapru was born in Srinagar in the state of Jammu & Kashmir in India, on April 22, 1958. He attended Burn Hall School, where he received the Higher Secondary degree in the Spring of 1975. He completed his Bachelor of Engineering degree in mechanical engineering from the Birla Institute of Technology and Science at Pilani in the Fall of 1980. He spent the next five years working with Britannia Industries Limited, a large manufacturer and marketeer of bakery products, in various production management capacities at different locations throughout India. He received his Master of Science degree in food engineering from the University of Massachusetts at Amherst in the Spring of 1988. He hopes to receive his Doctor of Philosophy degree in the Summer of 1991 in agricultural engineering from the University of Florida at Gainesville. He is married to Preevanda Sapru and has a two-year-old daughter, Khushboo.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Glen H. Smerage
Associate Professor of Agricultural Engineering

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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